EXHIBIT 1

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Oostman, Jr. et al.

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(45) **Date of Patent: Jan. 27, 2004**

(54) **FLUORESCENCE DETECTION INSTRUMENT WITH REFLECTIVE TRANSFER LEGS FOR COLOR DECIMATION**

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(73) Assignee: **Becton, Dickinson and Company**, Franklin Lakes, NJ (US)

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(22) Filed: **Aug. 28, 2001**

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(51) **Int. Cl.**[7] **G01N 21/64**
(52) **U.S. Cl.** **250/461.2**; 250/459.1
(58) **Field of Search** 250/461.2, 461.1, 250/459.1

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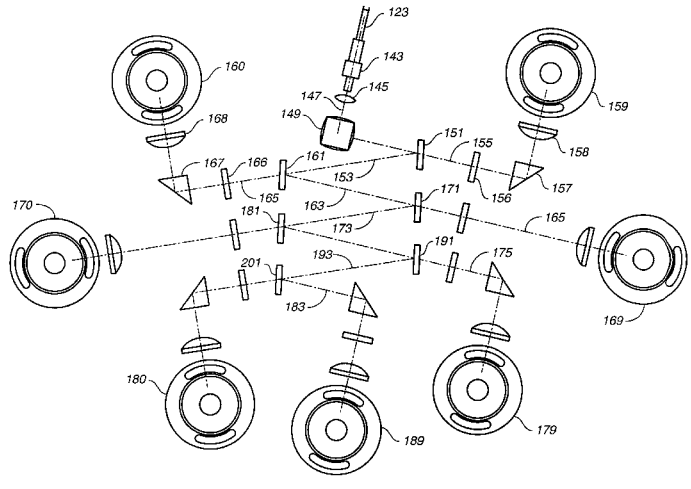
(57) **ABSTRACT**

An optical instrument using a plurality of lasers of different colors with parallel, closely spaced beams to stimulate scattering and fluorescence from fluorescent biological particulate matter, including cells and large molecules. A large numerical aperture objective lens collects fluorescent light while maintaining spatial separation of light stimulated by the different sources. The collected light is imaged into a plurality of fibers, one fiber associated with each optical source, which conducts light to a plurality of arrays of detectors, with each array associated with light from one of the fibers and one of the lasers. A detector array has up to ten detectors arranged to separate and measure colors within relatively narrow bands by decimation of light arriving in a fiber. A large number of detectors is mounted in a compact polygonal arrangement by using reflective transfer legs from multiple beam splitters where the transfer legs arise from a polygonal arrangement of beam splitters in a circumference within the circumferential arrangement of detectors.

37 Claims, 11 Drawing Sheets



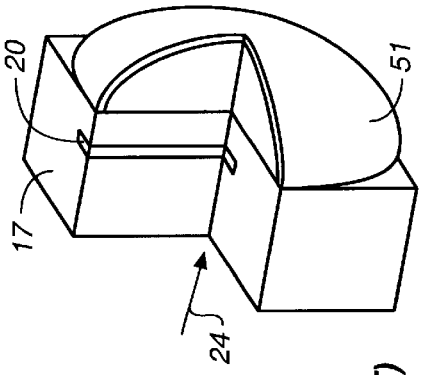


FIG._2
(PRIOR ART)

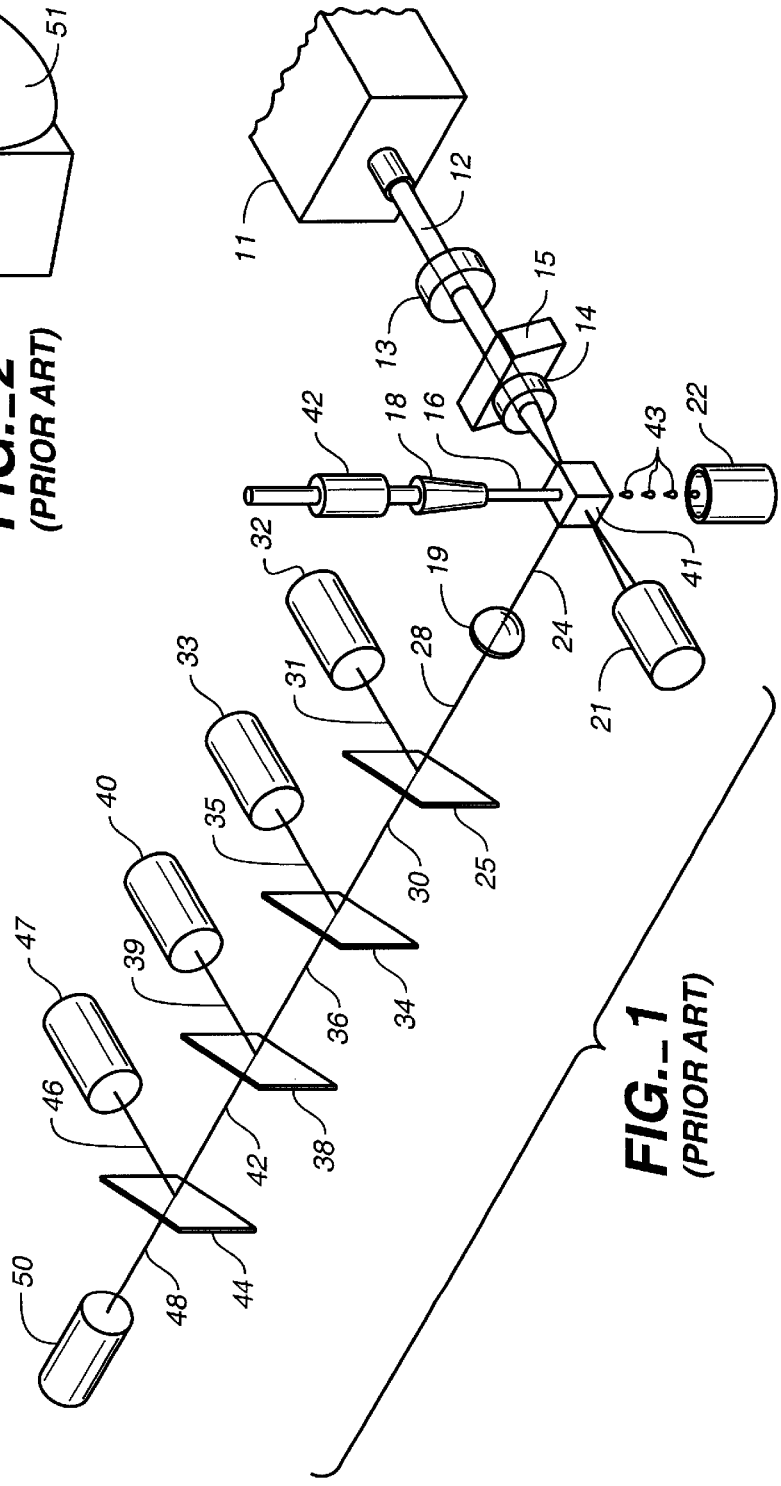


FIG._1
(PRIOR ART)

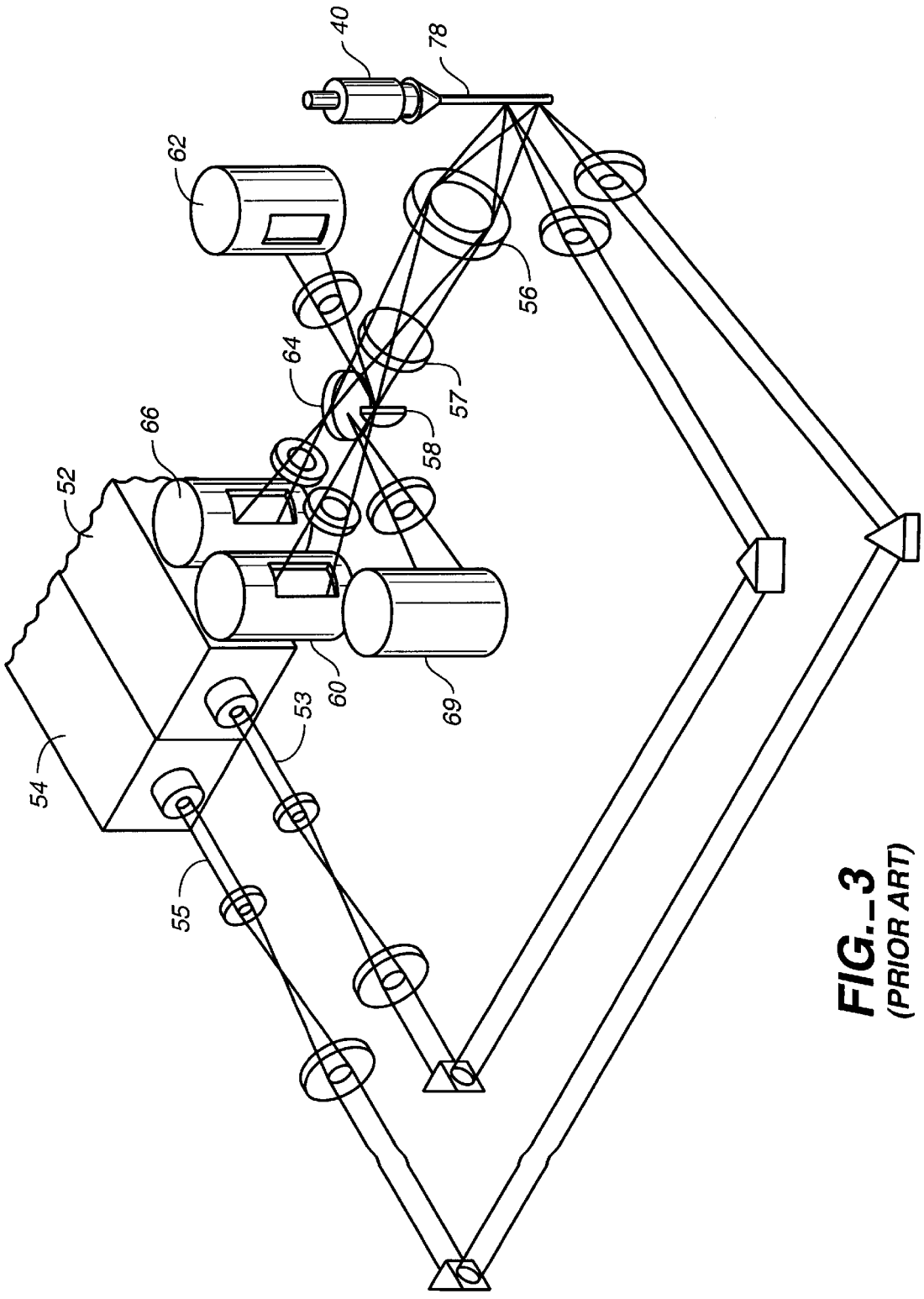


FIG._3
(PRIOR ART)

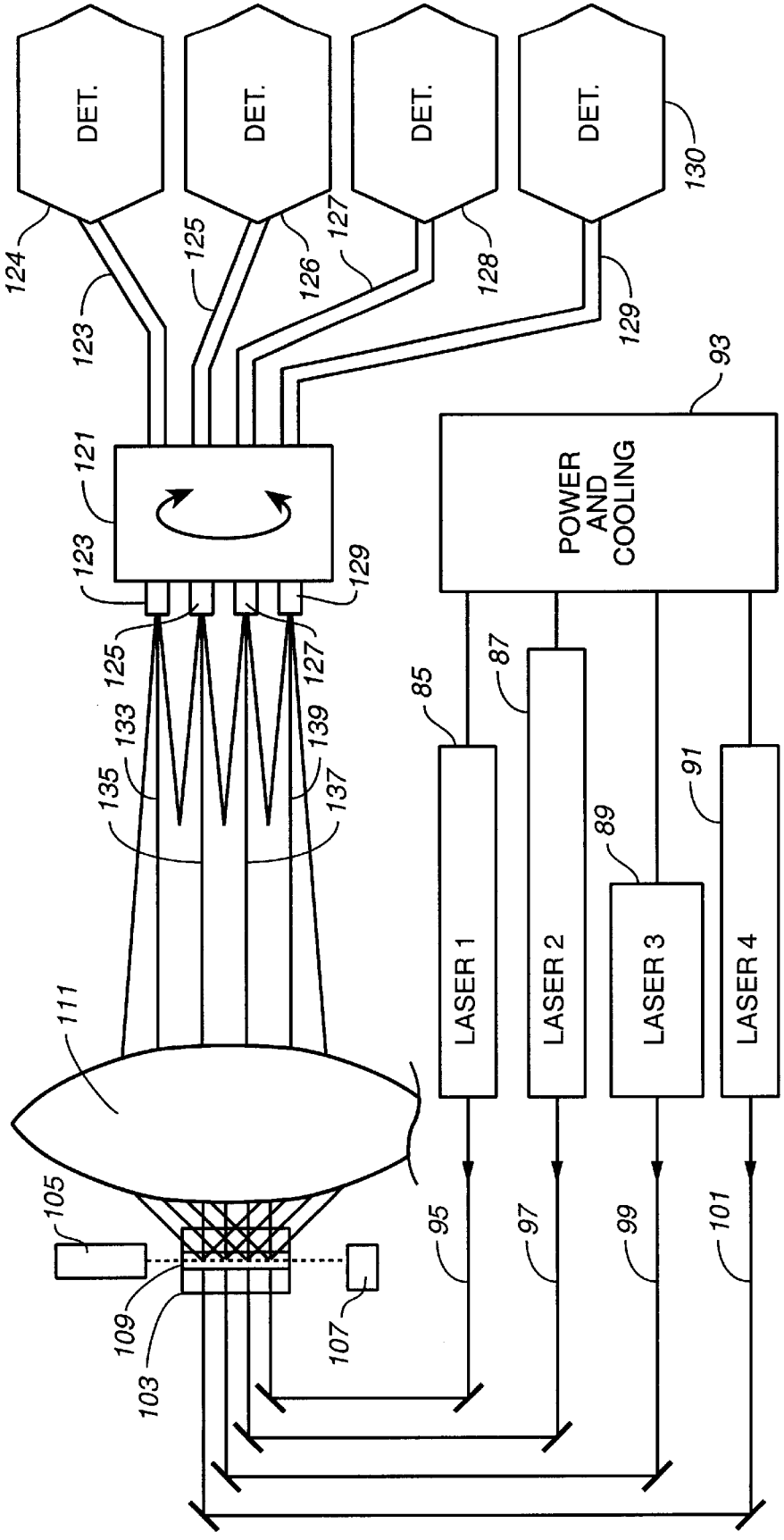

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FIG._4

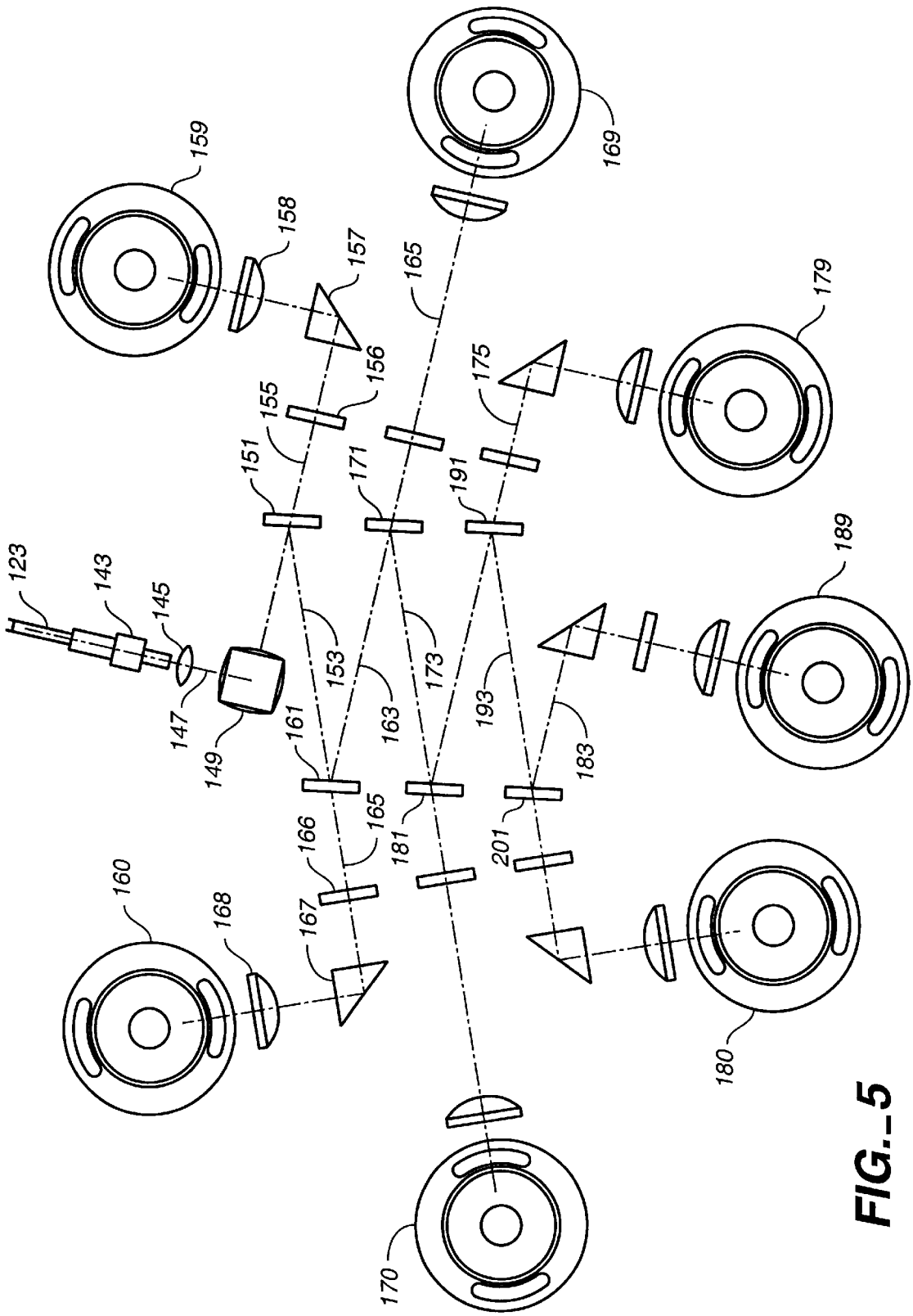
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FIG._5

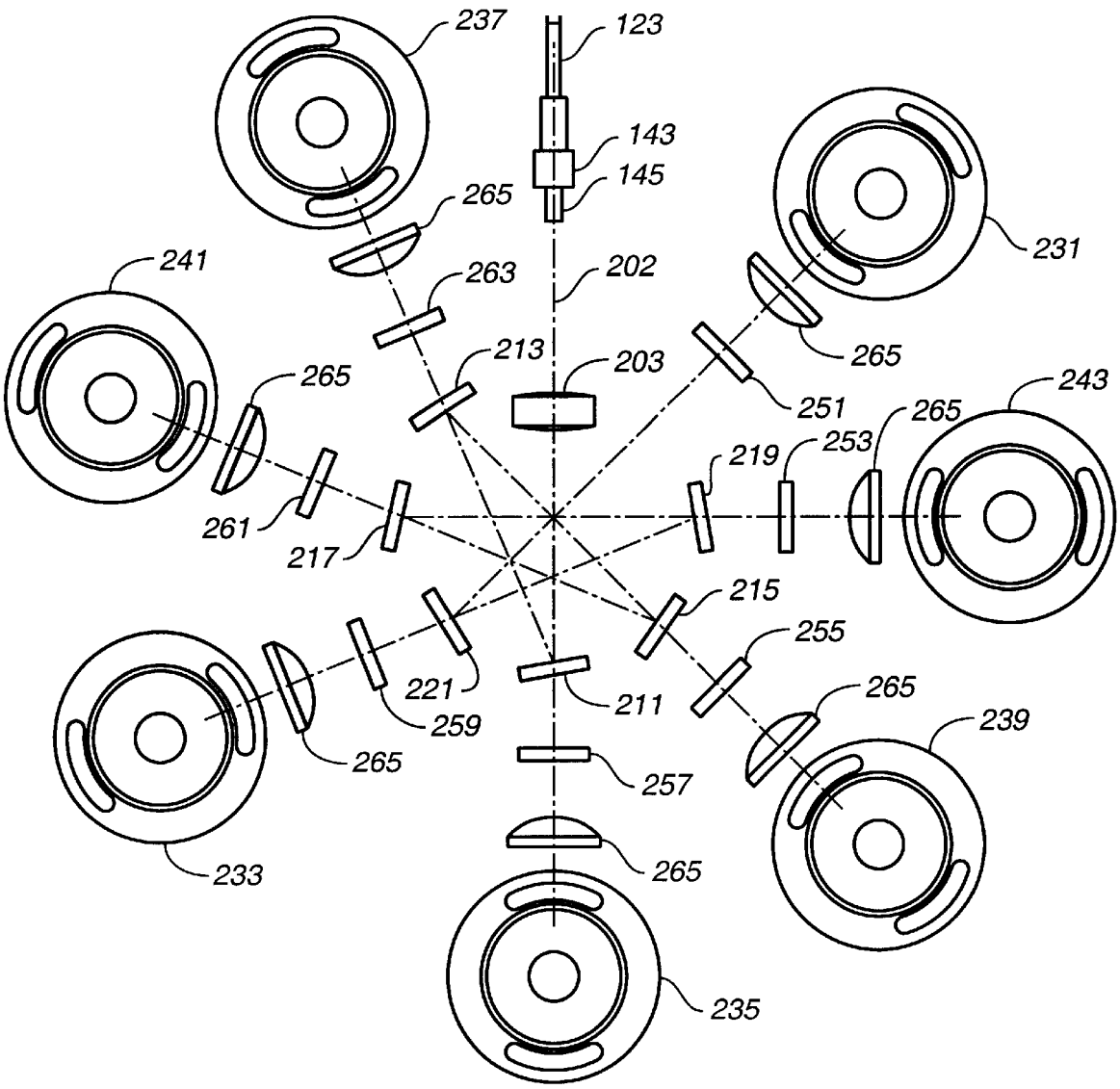
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FIG._6

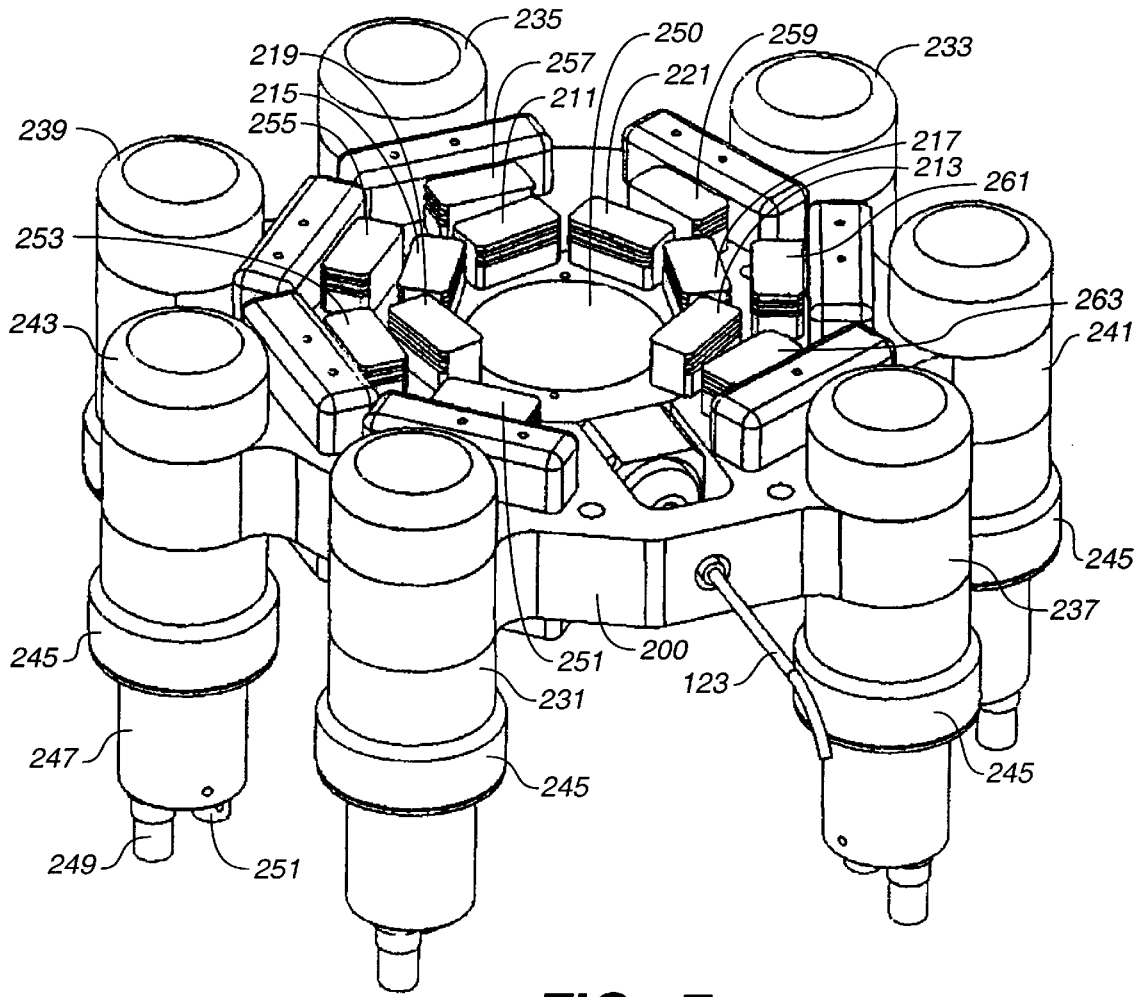
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FIG._7

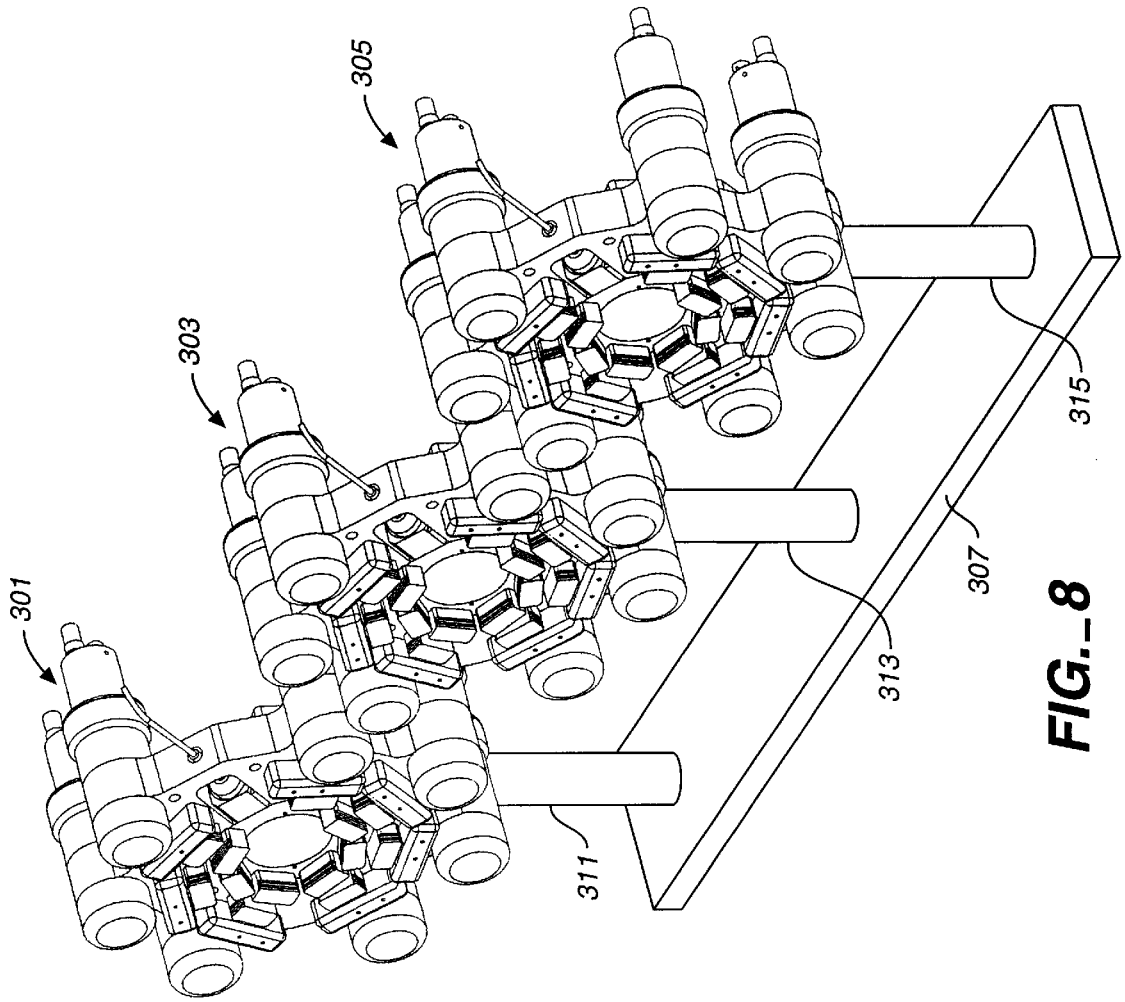

FIG._8

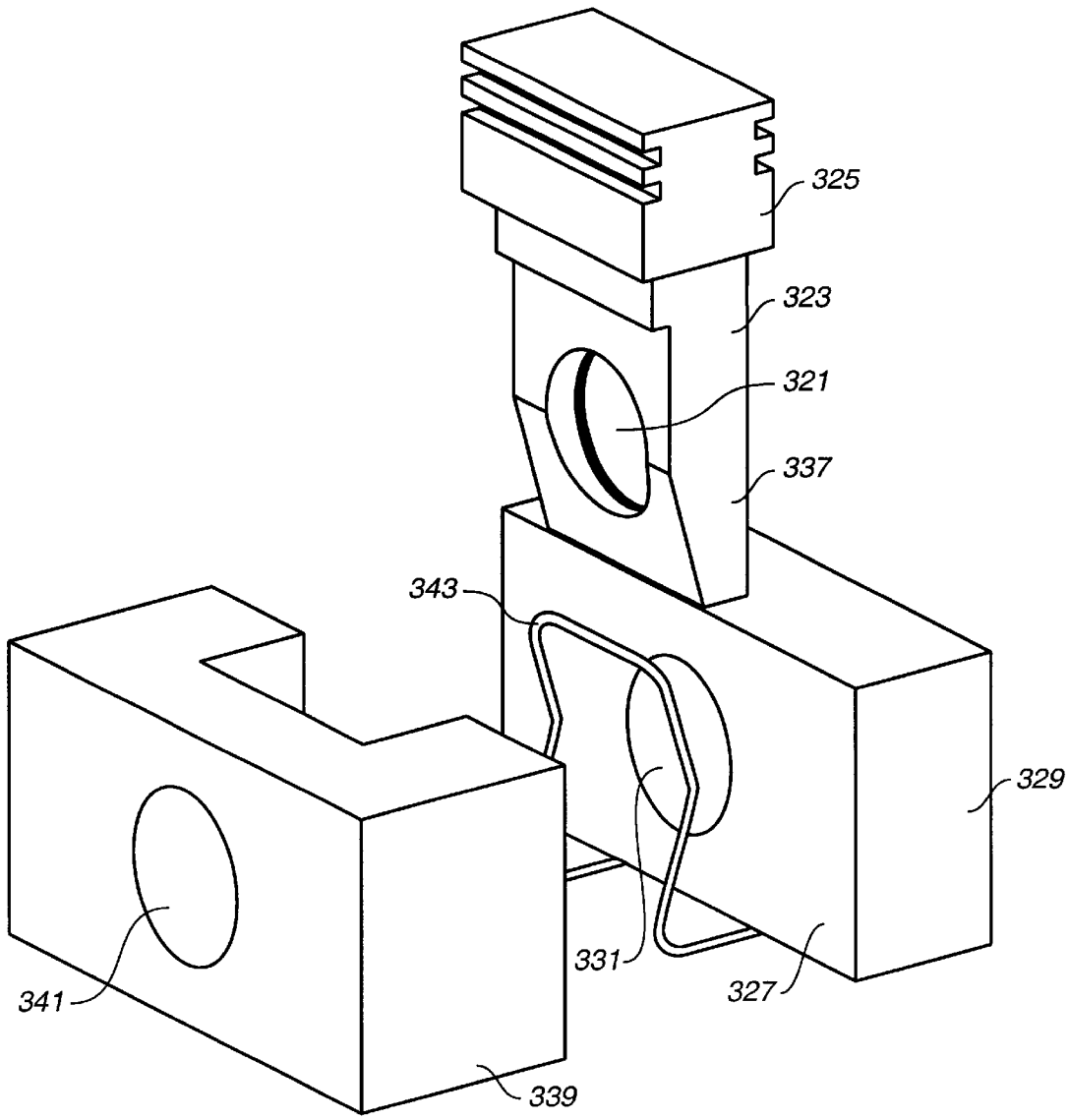

FIG._9

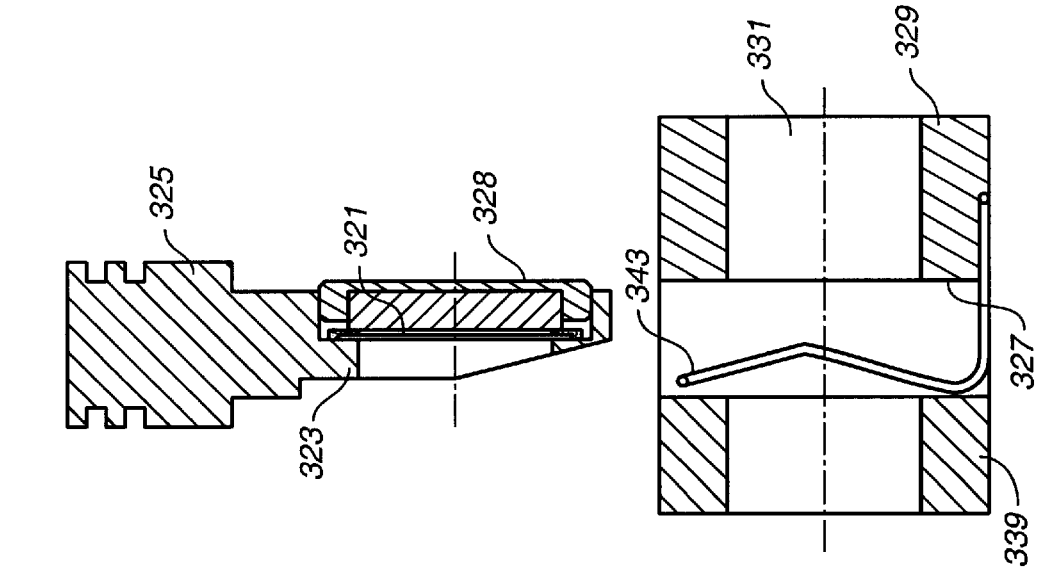

FIG._11

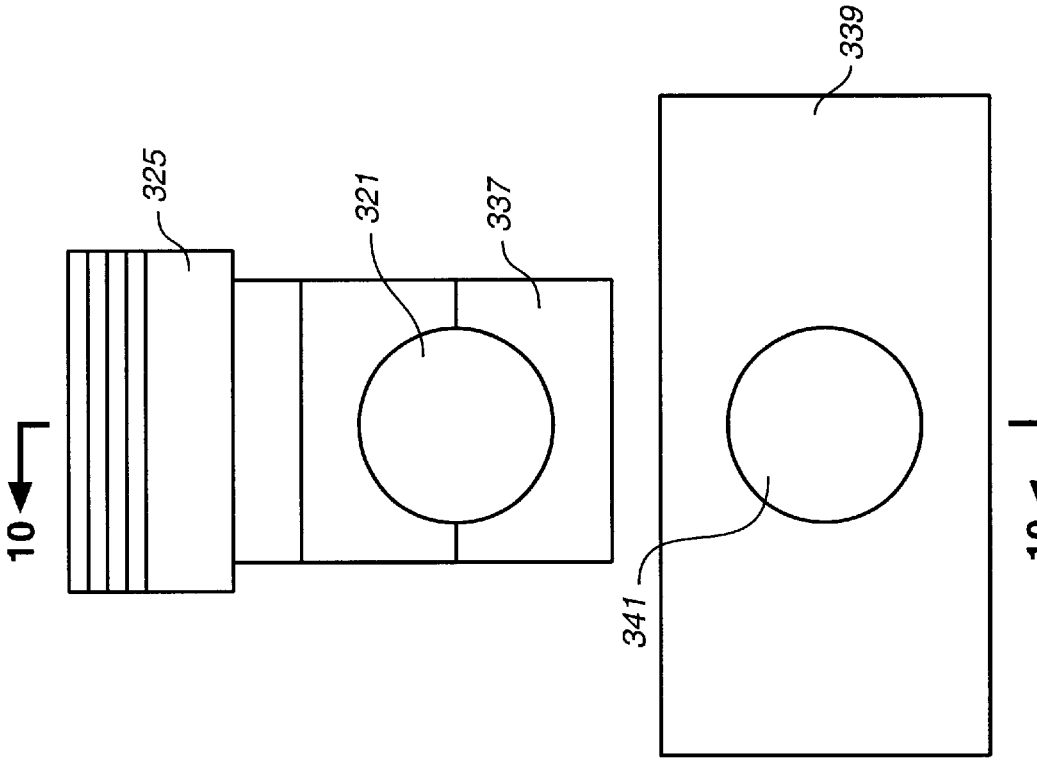

FIG._10

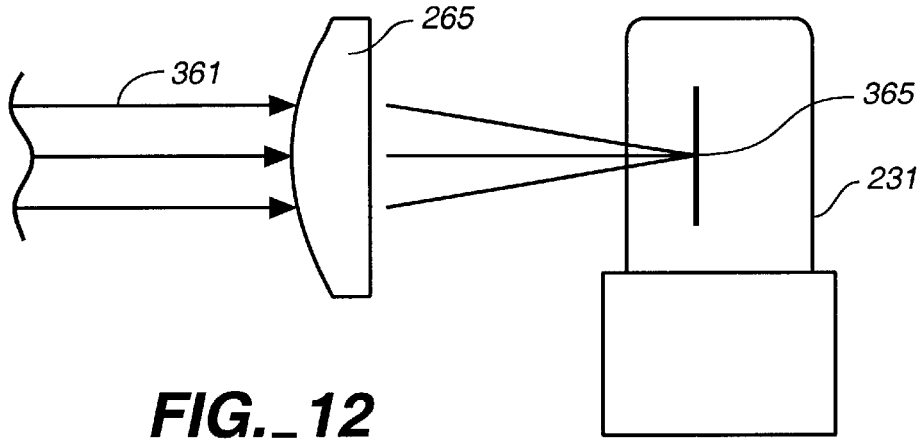

FIG._12

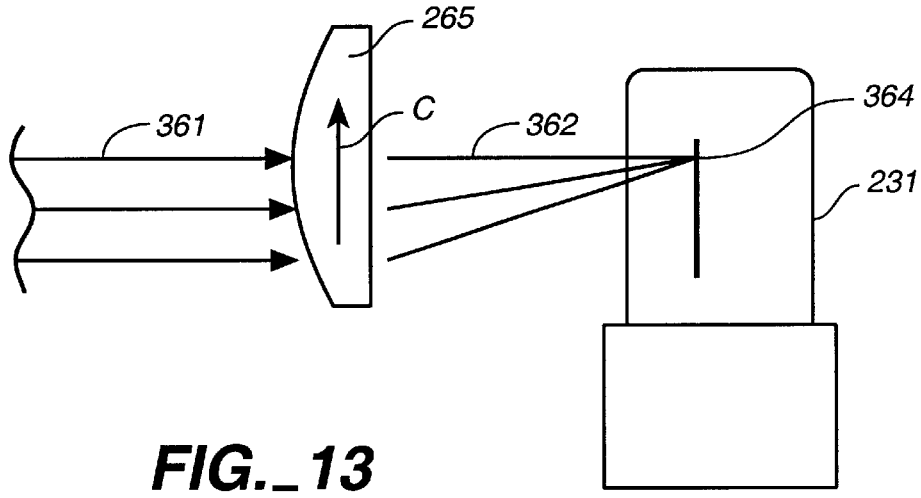

FIG._13

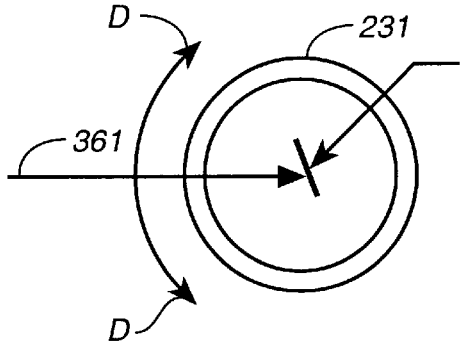

FIG._14

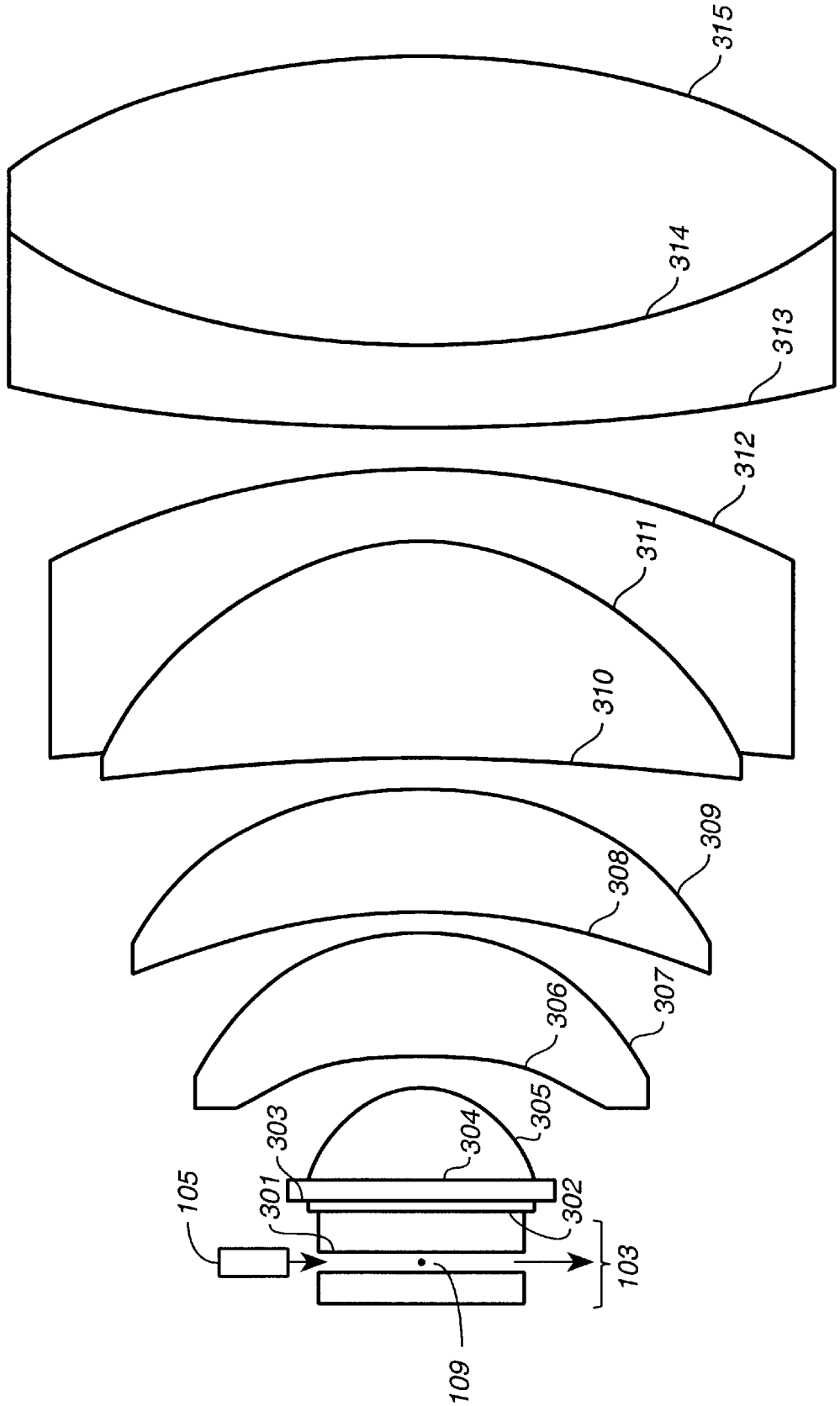
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FIG._15

FLUORESCENCE DETECTION INSTRUMENT WITH REFLECTIVE TRANSFER LEGS FOR COLOR DECIMATION

TECHNICAL FIELD

The invention relates to analytical instruments for flourescent light analysis from target specimens and, more particularly, to such an instrument employing increased color decomposition of fluorescent signals from target substances.

BACKGROUND ART

As an example of fluorescent light decomposition for bioanalytical studies, in high throughput screening, the ability to simultaneously detect a plurality of fluorescent dyes with good wavelength discrimination enables deeper multiplexing and higher throughput. In another example using fluorescent light analysis, simultaneous detection of multiple dyes associated with cells allows simultaneous assay of cell surface antigens, organelle states, nucleic acid assay, and intercellular protein content to be detected in a single assay. Multiple wavelength detection requires detectors which can separate many bands of colors. This has commonly been done using dichroic mirror beam splitters.

U.S. Pat. No. 5,317,162 to B. Pinsky and R. Hoffman, assigned to the assignee of the present invention, describes an instrument for phase resolved fluorescent analysis. The architecture of that instrument is similar to prior art instruments which rely upon color decomposition of a beam of fluorescent light derived from a laser impinging upon a fluorescent target. Such an apparatus is described in the book *Practical Flow Cytometry,* by H. M. Shapiro, Third Edition (1995), p. 9. The book describes an apparatus similar to what is shown in FIG. **1**. A laser beam **12** from an air cooled argon ion laser **11** is used to generate a fluorescent signal which is subsequently decomposed or decimated. The beam **12** passes through focusing elements **13**, **14** and **15** to impinge upon a fluorescent substance in a flow cell **41**. Fluorescent target material, such as fluorescently tagged cells or particles within a liquid stream **16** flow through the flow cell **41**. Particles **43** having passed through flow cell **41** are collected in container **22**. Flow is adjusted by a fluidic system **18** which provides a hydrodynamically focused flow of cells within a sheath fluid. As the target substance passes through the flow cell, the focused light beam **12** intersects the liquid stream, causing fluorescent excitation, including the scattering of light. A photodiode detector **21** is positioned to receive forwardly scattered light. The fluorescent light is typically collected at an angle which is 90° relative to the excitation access of the light beam **12**. Axis **24** represents the 90° viewing axis for collection of fluorescent light. Objective lens **19** is placed across axis **24** to collect and collimate the fluorescent signal from the target substance. Fluorescent light collected by the lens **19** is formed into a beam **28** which impinges upon the dichroic mirror **25**. The dichroic mirror reflects light above 640 nm and transmits the remainder as the transmitted leg **30**. Reflected leg **31** is directed to the red light fluorescence detector photo multiplier tube (PMT) having a 660 nm longpass filter. Detector **32** thus registers the red light component of the collected fluorescent signal from the flow cell **41**. The transmitted leg **30** impinges upon the dichroic mirror **34** which reflects light above 600 nm. The reflected leg **35** is orange light which is detected by the orange fluorescence detector PMT 33 having a 620 nm bandpass filter. The transmitted leg **36** impinges upon the dichroic mirror **38** which reflects light above 550 nm and transmits the remainder in transmitted leg **42**. Reflected leg **39** is detected by the yellow fluorescence detector PMT 40 having a 575 nm bandpass filter.

The transmitted leg **42** impinges upon dichroic mirror **44** which reflects light above 500 nm. The reflected leg **46** impinges upon the green fluorescence detector PMT 47, while the transmitted leg **48** consists of essentially blue light which is directed into the orthogonal scatter detector PMT 50 with a 488 nm bandpass filter, registering blue light. In this manner, the fluorescent signal in beam **28** collected by collector lens **19** is decomposed into five colors with the amplitude of each detector being recorded simultaneously to form a spectral characteristic of the fluorescent material illuminated by the laser beam.

The flow cell **41** is typically a flat-sided quartz cuvet of square or rectangular cross-section with a flow path therethrough. Such a quartz cuvet of the prior art is described in international patent publication WO 01/27590 A2, owned by the assignee of the present invention, shown in FIG. **2**.

In that international patent application, the flow cell mentioned above is described with an aspheric reflective light collector, unlike the lens **19** shown in FIG. **1**. The apparatus of the international patent application mentioned above is shown in FIG. **2** where a flow cell **17** is a quartz block having a flow channel **20** where a liquid stream containing fluorescent material is directed through the cell in a stream controlled by a nozzle. The flow cell of FIG. **2** has a reflective aspheric light collector **51** collecting light scattered to a side of the flow cell opposite the side where lens **19** is situated. An aspheric reflective element **51**, placed on the side of flow cell **17** opposite collector **19** serves to augment the light directed toward lens **19**, or in some cases performs the function of lens **19**. The reflective collector **51** is coated with a broadband reflecting material for augmenting the amount of light collected from the flow cell. The aspheric shape may be parabolic or ellipsoidal, having focal properties to match light collector **19** of FIG. **1**.

The apparatus of FIG. **3** is described in U.S. Pat. No. 4,727,020 to D. Recktenwald and assigned to the assignee of the present invention. This device shows a pair of lasers **52** and **54** directing light to a flow cell **78** so that two different illumination profiles may be used to illuminate a sample. Each laser is selected for stimulating the desired fluorescent emission from target substances. A set of detectors is associated with a different color band. For example, laser **52** generates a beam **53** impinging upon the flow stream **78** and producing a fluorescent signal collected by lens **56**, focused by lens **57** onto dichroic mirror **58**, a beam splitter, for analysis by detectors **60** and **62**. Similarly, laser **54** generates a beam **55** which impinges upon the flow which includes the particles under study in air and generates scattered fluorescent light, collected by light collector **56** and imaged by lens **57** onto dichroic mirror **64** where the beam is split between detectors **66** and **69**. In summary, it is known that groups of detectors can be associated with different lasers simultaneously illuminating the same target substance.

An object of the invention was to provide an improved system for detecting fluorescent light having multiple colors emitted from a target using a greater number of detectors than has been achieved in the prior art.

SUMMARY OF THE INVENTION

The above object has been achieved in an optical instrument having a detector arrangement featuring a larger num-

ber of spectrally diverse detectors than previously available. The detectors are fed by a plurality of lasers of different colors with parallel, spaced apart beams impinging upon fluorescent target material at different locations which may be in a channel, a plate, or the like. By using a plurality of lasers, a wide range of spectral responses may be stimulated from fluorescent target material. The target material may be fixed or flowing. Spatially separated fluorescence associated with each beam and emanating from the target material is collected by a large numerical aperture collector lens that preserves the spatial separation of the light originating from the plurality of sources, i.e. the fluorescent signatures of the laser beams on the target material is preserved. Fluorescent light stimulated by the different sources is imaged into a plurality of optical fibers that carry the light to separate detector arrays. Each array has a series of beam splitters in a series or cascade arrangement receiving light from an associated fiber and relaying part of the light to a downstream beam splitter, spectrally filtering the light on each relay within the cascade arrangement by means of coatings associated with the splitters. Within each array, light reflected from a beam splitter is forwarded to a downstream splitter, while light transmitted through a beam splitter is sent to a detector. This means that the reflected component is a broadband wavelength component and the transmitted component is filtered to be a narrowband wavelength component. For the last beam splitter, light from the reflective leg may be sent to a detector, as well as light from the transmitted leg. Since, for most optical coatings on a beam splitter, the fraction of light reflected from a beam splitter exceeds the transmitted fraction, the downstream beam splitters receive more light from the reflective transfer legs than the prior art arrangement where downstream beam splitters receive light from the transmitted transfer leg. Each array of detectors is arranged in a polygonal compact cluster. The detector configuration of the present invention is modular because light from each laser is spatially separated from other lasers and each detector cluster has at least 6 detectors. The clusters may be physically separated since optical fibers can feed light to clusters in remote locations or in stacks or racks. In this instrument, collected light is transmitted to a plurality of beam splitters. Note that the beam splitters, split light into a transfer leg and a transmitted detector leg, as in the prior art. However, unlike the prior art, the transfer leg is reflected from beam splitters and forwarded to another beam splitter and the transmitted detector leg is directed to a detector. This is true for a majority of beam splitters, but not for the last one receiving a maximally attenuated transfer leg where the transfer leg is either sent to a detector or terminated. So the last dichroic mirror may be associated with two detectors, one for the reflected leg and one for the transmitted leg. By using reflective transfer legs for most detectors, the detectors may be clustered in a polygonal arrangement of between five and ten light detectors in a common plane. Here, the term "most detectors" refers to all transfer legs except the last one, but is not limited to the last leg.

By maintaining spatial separation for the input beams, spatial separation can be preserved in the output transfer beams, with each transfer beam directed into an optical fiber for delivery to a detector cluster. This allows detector clusters to be stacked or placed in racks, with optical fibers carrying transfer beams to the location of an input port of each cluster. Once inside of a cluster, the transfer beam is decimated by the dichroic beam splitters, each beam splitter inclined to a transfer leg at a preferred angle centered on 11.25 degrees. Other angles will work but not as efficiently. Each beam splitter achieves color separation in the usual way, i.e. by transmitting light of a particular wavelength. This transmitted light is directed to a photomultiplier tube, or the like, which is positioned, to the extent possible, to detect light in the transmitted detector legs associated with the split beam. A focusing lens and the detector photomultiplier tubes are positionally relatively adjustable so that an optimum detector position can be found by motion of a detector element relative to a lens focusing incoming light. In this manner, the fluorescence associated with each of several laser beams is simultaneously decimated into bands characteristic of the target material within the detector array of each cluster. A group of clusters provides color decimation much greater than heretofore available. Moreover, the apparatus is modular because a greater number of fibers can feed a greater number of clusters. One of the advantages of using a reflective transfer leg to relay the optical signal for decimation, rather than the transmitted leg, is that the reflective transfer leg is a stronger optical signal. After encounters with several beam splitters, the signal attenuation in a relayed reflective transfer leg signal is substantially greater than for an optical signal in which the relay was transmitted through an equal number of beam splitters, as in the prior art.

In one embodiment, the light collection and detection optics are included in a system having a plurality of lasers producing input beams of different wavelength profiles to simultaneously illuminate a fluorescent target, usually fluorescent particulate matter which could be discrete small particles, including cells, or large biological molecules. The term "color decimation" refers to the simultaneous spectral breakdown of polychromatic light beams from a target substance into narrow bands of light arriving at detectors. Scattered light is measured by other detectors not relevant to this invention or this application. Scatter detectors are not described herein. Collection of fluorescent light is by a lens similar to a microscope immersion lens of large numerical aperture, the lens forming output transfer beams directed to a plurality of dichroic mirrors. After collection, the light is imaged into fibers, then distributed to "n" clusters of "m" detectors, yielding an "n" times "m" number of detectors resolving the fluorescent light stimulated by the input beams. Each cluster isolates light within the corresponding array of detectors. Clusters may be mounted on rails, racks or stacks in a modular arrangement.

DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective plan view of a multi-color flow cytometer with single wavelength excitation in accordance with the prior art.

FIG. **2** is a perspective view of a light collector of the prior art for use with a flow cytometer of the kind illustrated in FIG. **1**.

FIG. **3** is a perspective view of a multi-color flow cytometer with plural wavelength excitation and a polygonal arrangement of detectors in accordance with the prior art.

FIG. **4** is a plan view of a multi-color optical instrument of the present invention.

FIG. **5** is a top plan of a planar polygonal detector arrangement showing decimation of an incoming beam with reflective transfer leg beam splitters in accordance with the present invention, the incoming beam received from a fiber bundle illustrated in FIG. **4**.

FIG. **6** is a top plan of an alternate planar polygonal detector arrangement showing decimation of an incoming beam with reflective transfer leg beam splitters, as in FIG. **5**

but with the beam having a folded path for compact placement of the detectors.

FIG. **7** is a perspective assembly view of the apparatus of FIG. **6**.

FIG. **8** is a perspective plan view of three detector arrays of the kind shown in FIG. **7** mounted on a rack in accordance with the present invention.

FIG. **9** is a perspective plan view of a detail of a mirror holder used in the apparatus of FIG. **7**.

FIG. **10** is a front elevation of the mirror holder illustrated in FIG. **9**.

FIG. **11** is a side sectional view of the mirror holder of FIG. **10**, taken along lines **10**—**10**.

FIG. **12** is a side plan view of a detector photomultiplier tube and optics used in the detector arrangements shown in FIGS. **5** and **6**.

FIG. **13** is a side plan view of a motion to optimize the sensitivity of the detector shown in FIG. **12**.

FIG. **14** is a top plan view of a motion to optimize the sensitivity of the detector shown in FIG. **13**.

FIG. **15** is a plan view of a light collector lens for use in the instrument input end arrangement illustrated in FIG. **4**.

BEST MODE FOR CARRYING OUT THE INVENTION

With reference to FIG. **4**, a first laser **85**, a second laser **87**, a third laser **89**, and a fourth laser **91**, all produce light with unique wavelength profiles and all are connected to respective power supplies and a cooling module **93**. The lasers emit respective beams **95**, **97**, **99** and **101** which are directed by means of beam-turning mirrors toward flow stream **103** causing the beams to intersect with the stream. Although the preferred embodiment features a flow cytometer, this instrument is merely illustrative of instruments which employ fluorescence detection and color separation. Other instruments include microscopes, electrophoresis instruments, spectrophotometers, and the like. The scope of the present invention is therefore not limited to flow cytometers.

A fluidic system **105** feeds tagged target liquid substances into a stream **103** in a controlled manner. Material which passes through the beam illuminated zone is collected in collection cup **107**. The illuminated zone is established by the four laser beams that impinge upon fluid tagged target material, thereby causing scattering and fluorescence. Each beam has a characteristic color produced by different types of lasing material. For example, characteristic laser illumination wavelength profiles may be produced by CO_2 lasers, argon ion lasers, copper vapor lasers, and helium neon lasers. Other colors are available from different types of lasers. The output power of each laser is typically between 10 and 90 milliwatts. At such power levels, a sufficiently strong optical signal is produced without damaging coatings on the surfaces of mirrors, fibers or beam splitters. Coatings are selected to achieve desired passband transmissions and may be specified from coating manufacturers.

Light of different colors intersects the flow stream **103** and interacts with fluid sample causing scattering and fluorescence that is spatially separated along a line parallel to the flow channel **109**. Scattered light can be processed by well-known scatter detectors. For simplicity, this description deals only with fluorescent light. This light appears to be originating at four spaced apart point sources or spots, each of which is imaged by a lens light collector **111** to four respective optical fibers **123**, **125**, **127** and **129**, all held in place by a movable holder **121** which securely mounts the fibers and allows both rotational and axial adjustment of the fibers relative to light collector **111**. In other words, the holder **121** may be moved so that the fibers optimize the input light into the fibers from collector **111**. The focal spots produced by collector **111** enter the tip of each respective fiber **123**, **125**, **127** and **129**, each of which is a multi-mode fiber.

Light collector **111** is a group of lens elements which is described with reference to FIG. **15**. The collector is placed very close to the flow stream, within a few millimeters. The distances shown in FIG. **4** are not to any scale and are out of proportion. This collector gathers fluorescent light from the fluorescent target material. The collector lens is a microscope objective lens similar to the fluid emersion microscope objective lens shown in U.S. Pat. No. 5,805,346, except that the lens of the present invention is a positive meniscus lens, while the fluid emersion microscope objective lens of the '346 patent is a negative meniscus lens. Other differences exists, but the lenses are similar in the number of optical elements and their arrangements. Various supports may be used with the goal of reducing vibration and allowing proper alignment of optical elements along the optical axes **133**, **135**, **137**, and **139** defined by collector **111**. The optical axes are maintained by each of the fibers, although each of the fibers may be bent to remove light to the location of a detector array. Light in each of the fibers **123**, **125**, **127** and **129** is transmitted to a respective detector cluster **124**, **126**, **128** and **130** which houses an array of detectors. Each array processes fluorescent light which has maintained spatial separation, i.e. color independence, to a large extent. In other words, the fluorescence stimulated by a particular laser has been preservedand forwarded to an array of detectors which operates independently from other arrays of detectors. Each array of detectors differentially separates bands of light by filtering, using coatings on beam splitters and lenses, in a known manner. With each cluster having between 3–4 and 10 or more detectors, each detector receiving a passband of between 10 and 75 nanometers, the instrument of the present invention has a wide spectral response.

Details of a detector array or cluster are shown in FIG. **5**. The optical fiber **123** has a fiber terminal **143**, which allow emergence of light and formation of a beam by means of a collimating lens **145**. The output beam **147** is directed toward a beam-turning mirror **149** directing light at a first beam splitting element **151** which is a dichroic mirror transmitting light in beam **155** in a transmitted detector leg toward a filter **156**, a beam-turning element **157**, and a focusing element **158** which directs the beam onto a light sensitive element of a photomultiplier tube **159**. The tube is adjusted so that its most sensitive area is exposed to the incoming beam. This beam is known as the transmitted detector leg because it is transmitted through the beam splitting element **151**. The beam splitting element has a coating which allows transmission of one band of optical signals, while reflecting light in another band in the form of beam **153** which forms a reflective transfer leg for the light which was originally in beam **147**. The reflective transfer leg **153** is seen to fall upon another beam splitting element **161** which is a dichroic mirror having different optical characteristics from the beam splitter **151**. The colors removed by beam splitter **161**, as well as the other beam splitters, are different, each beam splitter removing a selected band of colored light in the same manner, but different wavelengths, as in beam splitters of the prior art described with reference to FIG. **1**.

Beam splitter **161** has a reflective transfer leg **163** reflected from the surface of the splitter, as well as a

transmitted detector leg **165** transmitted through the beam splitter to the filtering element **166**, the beam-turning element **167** and the focusing element **168**. The transmitted beam impinges upon a sensitive portion of detector **160** where the amount of light associated with the band transmitted by beam splitter **161** is measured. In the same manner, beam splitters **171**, **181**, **191** and **201** split incoming beams which are light beams reflected from upstream beam splitters, with beam splitter **191** being upstream of beam splitter **201**, beam splitter **181** being upstream from beam splitter **191**, and beam splitter **171** being upstream from beam splitter **181**, etc. Each beam splitter, except for the last one, beam splitter **201**, separates light into a reflective transfer beam, with the transfer beam **173** being reflected from beam splitting dichroic mirror **171** and the transmitted beam through dichroic mirror **171** being beam **165** impinging upon detector **169**. On the other hand, the reflected beam **173** is transmitted to beam splitter **181**, with the transmitted leg being directed to detector **170**. Beam **175**, passing through the beam splitter **191**, is directed to a detector **179** while the reflected leg **193** goes to the last beam splitter, namely dichroic mirror **201**. This element, unlike the other beam splitters, has two detectors associated with it. One detector **180**, receives light transmitted through the beam splitter **201** toward detector **180** while light reflected from beam splitter **201** forms a reflected leg **183** which impinges upon detector **189**. In this manner, all of the detectors illustrated in FIG. **5** form an array which decimates light from a single fiber **123**. As mentioned previously, the fiber **123** is associated with scattered and reflected light collected from one of the lasers mounted on the optical bench. Thus, for each laser there is an array of detectors in a cluster. In FIG. **5**, the transfer leg forwarded upstream from one beam splitter to the next follows a zigzag pattern. In FIG. **6**, the transfer legs intersect in a star-shaped pattern yielding a more compact polygonal arrangement of detectors.

In FIG. **6**, the optical fiber **123** is terminated in a terminal **143** directing an output beam **202** through a collimating lens **203** and thence onto an array of beam splitters **211**, thence to beam splitter **213**, then to beam splitter **215**, then to beam splitter **217**, then to beam splitter **219**, and, lastly, to beam splitter **221**. The arrangement of beam splitters is in a polygonal pattern. In each case, the transfer leg is reflective, with the beam splitter being a dichroic mirror which is inclined at an angle of 11.25° to perpendicular, i.e. a small angle, say between 5° and 20°. It has been found that this angle optimizes balance between reflection and transmission. On each bounce from a beam splitter, part of the beam called the "detector leg" is transmitted through the beam splitter toward one of the detectors. Detector **231** is associated with the transfer leg of beam splitter **221** while the detector **233** is associated with the detector leg from the same beam splitter. Detector **235** is associated with the detector leg coming through beam splitter **211** while detector **237** is associated with the detector leg through beam splitter **213**. Detector **239** is associated with the detector leg through beam splitter **215** while detector **241** is associated with the transfer leg from beam splitter **215** and the detector leg through beam splitter **217**. Correspondingly, the detector **243** is associated with the transfer leg from beam splitter **217** and the detector leg through beam splitter **219**. Each of the beam splitters is a dichroic mirror having different wavelength characteristics for decimating the input beam **202** into different colors which register at the different detectors. Coatings applied to the dichroic mirrors account for reflection of some wavelengths and transmission of other wavelengths. Laser light of a particular frequency will stimulate fluorescent emission in generally known wavelength bands. Light in these bands is collected and passed through an optical fiber to a detector array, the detectors arranged in a polygonal pattern of greater circumference than the polygonal pattern of beam splitters. Within each cluster decimation of the light occurs, with passbands of 10–75 nanometers registering at each detector, depending on the sharpness of filtering of the coatings applied to the beam splitters. Additional selectivity of the signals reaching the detector may be gained by a series of filters **251**, **253**, **255**, **257**, **259**, **261** and **263**, the filters arranged in a polygonal pattern with a polygonal circumference greater than the circumference of the beam splitters but less than the circumference of the detectors. Each of these filters is placed in front of a corresponding detector. The filter has a bandpass over a range of wavelengths which is of particular interest in the corresponding detector. Associated with each detector is a focusing lens **265** for focusing light in a detector leg on a sensitive spot of the detector. Each lens **265** is movable for adjusting the focal spot during calibration of the instrument.

In FIG. **7**, a cluster with an array of detectors and an array of beam splitters is seen to be held in place by a frame **200** which generally supports detectors **237**, **241**, **233**, **235**, **239**, **243** and **231** in a polygonal array where the polygon is drawn connecting the axial centers of each of the cylindrical detectors, the detectors being photomultiplier tubes. Each tube is seated in a tube mount **245**. A tube connector base **247** makes contact with pins of each tube. An electrical feed-through **249** allows power to come to connector base **247** while signals exit the tube through another feed-through **251**. Similar connector bases and feed-throughs exists for each tube. Within the center of the frame **200** is a coverplate **250**. About a first close distance from the coverplate is a polygonal array of dichroic mirror holders for the dichroic mirrors **213**, **217**, **221**, **211**, **215** and **219**. A slightly further distance are the filter holders **263**, **261**, **259**, **257**, **255**, **253** and **251**. The dichroic mirror holders and the filter holders are mounted in vertically removable housings so that dichroic mirrors and associated filters may be interchanged or replaced.

In FIG. **8**, a plurality of clusters **301**, **303** and **305** is shown to be vertically mounted on a rack **307** by means of standoff supports **311**, **313** and **315**. The standoff supports are merely illustrative of the manner in which three arrays may be mounted on a rail or rack for easy replacement or modular supplementation. Each cluster is of the type shown in FIG. **7**.

In FIGS. **9–11**, the construction of a removable beam splitter holder is shown. The beam splitter mirror **321** is held in a mirror holder frame **323** at a desired angle. Frame **323** is supported by a block **325** having channels for finger contact in a non-slip manner. The mirror holder frame **323** has a flat side **328** which presses against the flat side **327** of a seating block **329** having a central aperture **331** corresponding to the position of mirror **321**. A facing block **339** has an aperture **341** in alignment with aperture **331** and with mirror **321**. The flat side **327** is a reference surface for positioning of the mirror **321**. A wire spring **343** serves to push the mirror holder frame **323** against the block **329**.

FIGS. **12–14** show how the transmitted leg of a beam **361** may impinge upon the focusing lens **265**. With motion of the focusing lens **265**, as shown in FIG. **13** in the direction of the arrow C, the focused beam **362** is moved to a more sensitive spot **364** on the photomultiplier tube **231** in comparison to a less sensitive location **365** shown in FIG. **12**. Additional sensitivity may be gained by slightly rotating the photomultiplier tube **231** with its housing, within the support frame to

optimize the output signal for a particular detector leg **361** focused on a detection element in the photomultiplier tube. Motion is indicated by the arrows D.

FIG. **15** shows the construction of light collector **111** in FIG. **2**. A flow cell **103** is shown at the left of the drawing with flow channel **109** and input fluid from fluidic system **105** passing through channel **109**. As has been previously noted, a flow system is but one type of optical instrument where fluorescence can be observed. Non-flow systems may also be employed with the detection apparatus of the present invention. A large numerical aperture ("N.A.") lens system, i.e. N.A. greater than one, positioned as shown in FIG. **4** is described according to the lens data contained in the following table. The numbered optical surfaces in the figure correspond to surface numbers in the leftmost column of the table. All radii and thickness values are in millimeters. Surface curvature tolerances for the lens data include 5 fringes for power (deviation of actual curvature from nominal curvature) and 1 fringe for irregularity (deviation from a perfect spherical surface). Tilt tolerance is 0.05 degrees from normal in any direction. Material tolerances are 0.0005 for refractive index and 0.8% for Abbe number.

Surface	Radius of Curvature	Thickness	Thickness Tolerance	Aperture Radius	Clear Aperture Radius	Material
109	4	0.0889	—	0.2		Water
301	4	1.94	—	4.6		Silica
302	4	0.1682	—	4.6		Gel
303	4	0.8	.025	5.1	4.6	BK7
304	4	3.915	.025	4.6	4.6	BK7
305	−4.66	1.5	.025	4.6A	4.6	Air
306	−16.918	5	.05	8.5	7.3	BK7
307	−10.894	1	.025	10	8.8	Air
308	−26.836	5	.10	11.5	10.2	BK7
309	−15.008	1	.025	12.5	11.1	Air
310	−103.704	9	.10	13	11.9	BK7
311	−14.012	3	.10	13	12.2	SF8
312	−34.38	2	.05	15.5	14.1	Air
313	+123.446	3	.10	17	14.9	SF8
314	+34.38	12	.10	17	15.1	BK7
315	−36.554	126.731	.50	17	15.5	Air

The lens proper (surfaces **303** through **315**) in this system is adapted to magnify and view cellular material within a cytometry flow cell or cuvette **103** (the flow cell inner and outer wall surfaces being optical surfaces **301** and **302** above). As indicated in the table, a flow cell has 0.007 inch (0.1778 mm) interior dimensions (wall-to-wall) and the fluorescent targets **109** to be detected and analyzed are immersed in saline water flowing through the cell **103**, nominally for lens design purposes through the center of the cell a distance of 0.0889 mm from the cell's inner wall. The 1.94 mm thick, fused silica, planar cell wall has a refractive index $_{nD}$ of 1.45857 and an Abbe number $_{vD}$ of 67.7. An optical gel layer provides an interface between the cytometry flow cell and the lens proper and improves lens mounting tolerances. The gel material is preferably NyoGel OC-431A sold by William F. Nye, Inc. of New Bedford, Mass., and has refractive indices at the 0.40 μm, 0.55 μm and 0.70 μm principal lens design wavelengths, respectively, of 1.487, 1.467, and 1.459. The gel should have a thickness less than 0.5 mm, and is selected in the above design to be 0.1682 mm thick. Other cytometry flow cells with different interior and wall dimensions, and other optical gels or oils could be used, with appropriate modifications in the lens specifications, optimized using commercially available software. Although lens positioning tolerances would be much tighter (0.025 mm or less), the lens could also be integrated with or mounted to the flow cell without using optical gel.

The lens glass types BK7 and SF8 (Schott glass designations) have been selected because they are relatively inexpensive stock materials that are easy to obtain in quantity, and because they are easy to grind and polish and don't stain easily. Other glass types could be used instead, including similar glass types from other optical glass suppliers, with appropriate modifications in the lens specifications. The optical glass designated BK7 [517642] has a refractive index $_{nD}$ of 1.51680 and an Abbe number $_{vD}$ of 64.17, and the optical glass designated SF8 [689312] has a refractive index $_{nD}$ of 1.68893 and an Abbe number $_{vD}$ of 31.18. All of the lenses in the preferred embodiment have spherical surfaces because they are inexpensive, more readily available in bulk, are more alignment tolerant, and are easier to assemble and test than aspheric lenses. However, if desired, modified lens specifications using one or more aspheric lenses have lower on-axis aberrations and could be used, although from a commercial standpoint the performance improvement likely would not be sufficient to justify their significantly greater expense and assembly difficulty.

The basic lens requirements include a numerical aperture of at least 1.17. (An object N.A. of 1.20±0.01 was used in obtaining the preferred embodiment that is set forth in the table above. A numerical aperture of 1.20 provides about 10 to 15% greater light collection than one of 1.17) The field of view should be at least 200 μm diameter and, if possible, as much as 400 μm or better. The present preferred embodiment has a field of view of 400 μm diameter. The working distance should be at least 1.75 mm, (2.2 mm is achieved in the preferred embodiment.) Most importantly, a lens system of less optical aberrations and high image quality is required for better resolution compared to existing cytometry lenses. In particular, the RMS spot size (a measure of resolution) in image space (for hypothetical point objects) for all wavelengths and all field points should be at most 100 μm. The present preferred embodiment achieves a calculated geometrical spot size of 85.04 μm at full field and of 71.86 μm on-axis. This puts a minimum of 80% of the optical energy of the image of an infinitely small point source within a circle of less than 200 μm diameter. This is a significant improvement over one existing cytometry lens design's 442.6 μm full field and 365.2 μm on-axis spot sizes and 800 μm diameter circle energy (at 80% energy).

Other design parameters for the lens optimization software include a magnification of at least 10X, and preferably between 10.5x and 11.5x, and a back focal length of 127±2 mm (as seen for surface **15** in the table, a back focal length of 126.731 mm is obtained for the present embodiment), and a wavelength range at least from 400 mm to 700 mm (the entire visible light range). The total length and lens barrel diameter should be as small as possible, i.e. less than 57 mm and 41 mm respectively, since space near the flow cell is in high demand in cytometry instruments. A lens length of 47.2 mm (combined thickness for surfaces **3** to **14**) and a maximum aperture radius (for less surfaces **13** to **15**) of 17 mm show that these size goals have been met.

The lens is seen to comprise (a) a nearly hemispheric plano-convex crown glass lens (surface **308** through **305** in the above table including the cemented plate of identical material added for handling) with its planar side **303** closest to the cytometry flow cell and its convex surface **305** having a radius of curvature in a range from 3.5 to 5.5 mm (4.66 mm in the present preferred embodiment); (b) a pair of positive

meniscus lenses (surfaces **306** to **309**) with their concave sides **306** and **308** closest to the flow cell (i.e. on the object side of the lens system) and with the surfaces **308** and **309** of the second meniscus lens being less sharply curved than the corresponding surfaces **306** and **307** of the first meniscus lens, which are in turn less sharply curved than the convex surface **305** of the plano-convex lens; and (c) a pair of positive doublet lens elements (surfaces **310–315**) to compensate for chromatic aberrations from the first three lens elements. The near hemispheric shape of the plano-convex lens (total axial thickness of the lens plus the attached plate of identical crown glass material being 4.715 mm compared to the 4.66 mm radius of curvature of the convex surface **305**, of difference of less than 1.2%) gives the lens system its large field of view. The convex radius of curvature range provides for a long working distance of at least 1.75 mm (about 2.2 mm in the present embodiment). Use of two meniscus lenses, and also the use of crown glass material (refractive index less then 1.55) for both the meniscus lenses and the plano-convex lens, reduce aberrations, which are generally proportional to the square of the amount of light bending at each refractive surface. The lower aberrations provide improved resolution, as indicated above the image spot size and circle energy. The doublets are not achromats themselves, but are over compensated so that the chromatic aberrations are reduced for the entire lens system.

What is claimed is:

1. An optical instrument for fluorescence analysis of many colors from a target having fluorescent material comprising,

a plurality of lasers of different wavelengths which generate a plurality of beams, said beams impinging upon fluorescent material,

a light collector to collect fluorescent light from said fluorescent material into an output transfer beam,

a plurality of dichroic mirrors arranged to receive the transfer beam from the light collector, said dichroic mirrors having a partially reflective surface splitting the light into a transfer leg and a transmitted detector leg, a majority of the dichroic mirrors receiving light from the partially reflective surface of another dichroic mirror, and

a plurality of detectors, with one detector being associated with a dichroic mirror, to receive light from the detector leg thereof.

2. The apparatus of claim **1** wherein the plurality of dichroic mirrors and the plurality of detectors is divided into a multiplicity of groups of mirrors and detectors and light from the light collector is directed into a multiplicity of fibers, each fiber delivering light to a group of mirrors and detectors.

3. The apparatus of claim **2** wherein each fiber is associated with collected light from a selected laser.

4. The apparatus of claim **2** wherein each of the groups of mirrors and detectors is arranged in a cluster.

5. The apparatus of claim **4** wherein each cluster lies in a plane mounted parallel to another cluster.

6. The apparatus of claim **1** wherein said light collector is an immersion objective lens.

7. The apparatus of claim **1**, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output transfer beam at an angle of 20° or less.

8. The apparatus of claim **1**, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output transfer beam at an angle between 5° and 20°.

9. An optical instrument for fluorescence analysis of many colors from a target substance comprising,

a plurality of beam sources having beams impinging upon fluorescent target material thereby producing fluorescent light,

a plurality of optical fibers corresponding in number to the plurality of beam sources, the fibers having associated optics collecting the fluorescent light, each fiber having an input terminal and an output terminal with an optical axis therebetween and projecting outwardly from each output terminal,

a plurality of arrays of dichroic mirrors, with at least one dichroic mirror in each array disposed along each projected optical axis thereby receiving light from a fiber output terminal, and

a plurality of detectors associated with each mirror array, with at least one detector associated with each mirror, the mirrors and detectors arranged in a single enclosure defining a cluster, thereby isolating the fluorescent light signatures of one cluster from another.

10. An optical instrument for fluorescence analysis comprising,

a holder supporting fluorescent targets,

a plurality of lasers directing beams to impinge upon the fluorescent targets thereby generating fluorescent light,

a light collector positioned to collect fluorescent light from targets in a manner maintaining separation of fluorescent light output for each incident beam,

a plurality of optical fibers, each fiber having an input end receiving output fluorescent light from the light collector and an output end directing said fluorescent light outwardly along a projected optical axis,

a series of dichroic mirrors associated with each projected optical axis, separating fluorescent light into output beams of constituent wavelengths,

a series of detectors intercepting the output beams of constituent wavelengths with a detector associated with each dichroic mirror, with a number of mirrors and detectors forming a cluster wherein light from only one fiber is in each cluster, a group of clusters associated with all of the optical axes.

11. The apparatus of claim **10** wherein said group of clusters is formed in the same horizontal plane.

12. The apparatus of claim **10** wherein said group of clusters is stacked.

13. The apparatus of claim **10** wherein said group of clusters is arranged in a rack.

14. The apparatus of claim **10** wherein said fibers correspond in number to the plurality of lasers.

15. The apparatus of claim **10** wherein said detectors are photomultiplier tubes.

16. The apparatus of claim **10** wherein said detectors are semiconductors.

17. The apparatus of claim **10** wherein said detectors have variable sensitivity over a detector area, the detectors generating an electrical signal representing the variable sensitivity of the detectors, the detectors and an intercepted beam being relatively moveable, whereby a selected sensitivity may be obtained.

18. The apparatus of claim **17** further defined by a series of focusing lenses associated with each dichroic mirror intercepting the output beams of constituent colors and directing light therefrom to (i) the detectors of variable zonal sensitivity and (ii) a series of focus lenses intercepting the output beams of constituent colors and directing light to the detectors of variable zonal sensitivity wherein relative motion of each focus lens and detector varies the sensitivity of the generated electrical signal.

19. The apparatus of claim 10 wherein said detectors are arranged in a polygonal array.

20. The apparatus of claim 10 wherein the number of detectors exceeds 5.

21. An optical instrument of the type having one or more input beams impinging upon fluorescent material to be analyzed with fluorescent light collected by a light collector and formed into at least one output beam for analysis, the output beam having a projected optical axis and a plurality of spaced apart dichroic mirrors disposed along the projected optical axis in a manner separating light at each mirror into a reflected beam and a transmitted beam, wherein one of the reflected and transmitted beams is a transfer leg carrying the beam further to the next dichroic mirror and another leg carrying light to a detector wherein the improvement comprises,

an arrangement of the plurality of dichroic mirrors wherein a majority of the dichroic mirrors receives light from a reflected beam coming from another dichroic mirror.

22. The apparatus of claim 21 wherein the number of dichroic mirrors is at least four.

23. The apparatus of claim 21 wherein all of the dichroic mirrors except one receive light from a reflected beam coming from a dichroic mirror.

24. The apparatus of claim 21 wherein all of the dichroic mirrors except two receive light from a reflected beam coming from a dichroic mirror.

25. The apparatus of claim 21, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output beam at an angle of 20° or less.

26. The apparatus of claim 21, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output beam at an angle between 5° and 20°.

27. An optical instrument for fluorescence analysis of many colors comprising,

a light transmissive holder supporting fluorescent target material,

a plurality of lasers of different colors having closely spaced beams illuminating said target material,

a light collector means for forming beams of fluorescent light with spatial separation, one beam associated with each laser,

a plurality of optical fibers, each fiber receiving light of a different color from one of the beams associated with each laser,

a plurality of detector arrays, each array forming a cluster receiving light from a fiber, each cluster having color decimation means for separating and detecting colors received from the associated detector cluster.

28. The apparatus of claim 27 wherein each color decimation means with a detector array has a plurality of beam splitters arranged in cascade relationship, at least one detector associated with each beam splitter, with a first beam splitter positioned to receive light from an associated fiber, the first beam splitter and most cascaded beam splitters having a reflective transfer leg of light and a transmitted transfer leg of light directed to an associated detector.

29. The apparatus of claim 28 wherein the detectors are arranged in a polygonal pattern having a first circumference.

30. The apparatus of claim 29 wherein the beam splitters are arranged in a polygonal pattern having a second circumference smaller than the first circumference.

31. The apparatus of claim 30 wherein a plurality of filters is arranged in a polygonal pattern, with a filter associated with each detector, the polygonal circumference of the filters greater than the circumference of the beam splitters but less than the circumference of the detectors.

32. The apparatus of claim 28 wherein each beam splitter is mounted in a removeable aligning holder.

33. The apparatus of claim 27 wherein each cluster receives light from the light collector by means of one of said optical fibers, the light in each optical fiber associated with fluorescence from a different laser.

34. The apparatus of claim 27 wherein said light collector means is a microscope immersion objective type of lens.

35. The apparatus of claim 34 wherein said lens has a numerical aperture greater than one.

36. The apparatus of claim 28 wherein the reflective transfer legs cross each other.

37. The apparatus of claim 28 wherein the reflective transfer legs do not cross each other.

* * * * *

EXHIBIT 2

US007129505B2

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Oostman, Jr. et al.

(10) **Patent No.: US 7,129,505 B2**
(45) **Date of Patent: Oct. 31, 2006**

(54) **FLUORESCENCE DETECTION INSTRUMENT WITH REFLECTIVE TRANSFER LEGS FOR COLOR DECIMATION**

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(51) **Int. Cl.**
G01N 21/64 (2006.01)

(52) **U.S. Cl.** **250/458.1**

(58) **Field of Classification Search** 250/458.1, 250/459.1, 461.1, 461.2
See application file for complete search history.

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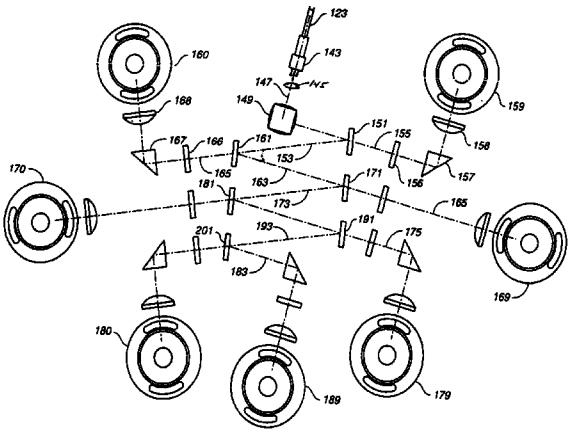
(57) **ABSTRACT**

An optical instrument using a plurality of lasers of different colors with parallel, closely spaced beams to stimulate scattering and fluorescence from fluorescent biological particulate matter, including cells and large molecules. A large numerical aperture objective lens collects fluorescent light while maintaining spatial separation of light stimulated by the different sources. The collected light is imaged into a plurality of fibers, one fiber associated with each optical source, which conducts light to a plurality of arrays of detectors, with each array associated with light from one of the fibers and one of the lasers. A detector array has up to ten detectors arranged to separate and measure colors within relatively narrow bands by decimation of light arriving in a fiber. A large number of detectors is mounted in a compact polygonal arrangement by using reflective transfer legs from multiple beam splitters where the transfer legs arise from a polygonal arrangement of beam splitters in a circumference within the circumferential arrangement of detectors.

11 Claims, 11 Drawing Sheets



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Steinkamp, J.A., et al, "Improved Method for Multicolor Fluorescence Detection Using Dichroic, Color-Separating Filters", Abstract 431C in, "Abstracts for the XIV International Meeting of the Society for Analytical Cytology", Journal of the Society for Analytical Cytology, Cytometry, Supp. 4 (1990).

* cited by examiner

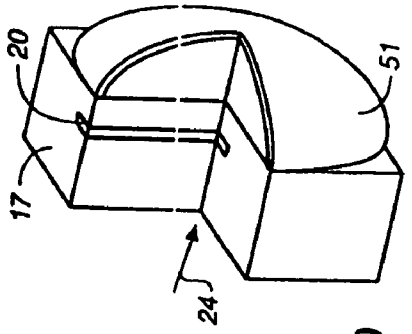


FIG._2
(PRIOR ART)

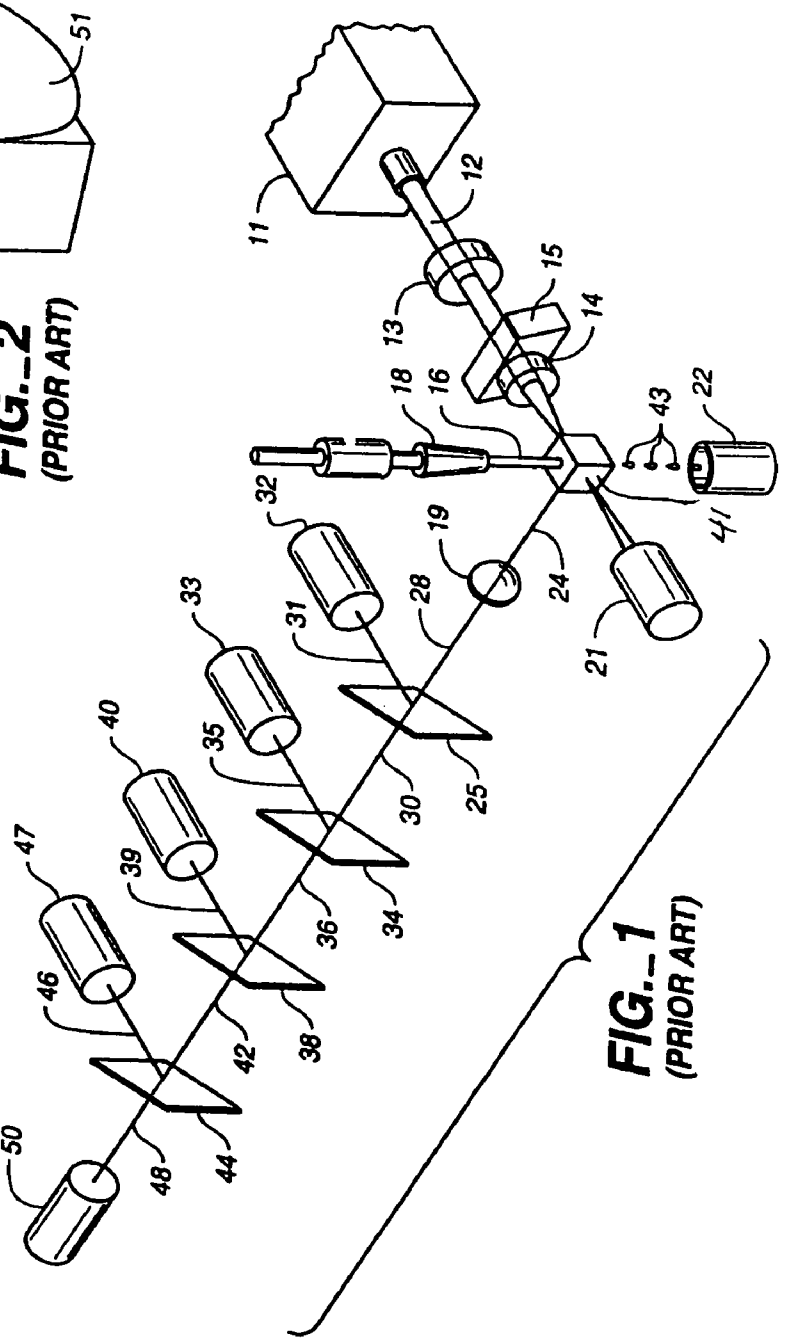


FIG._1
(PRIOR ART)

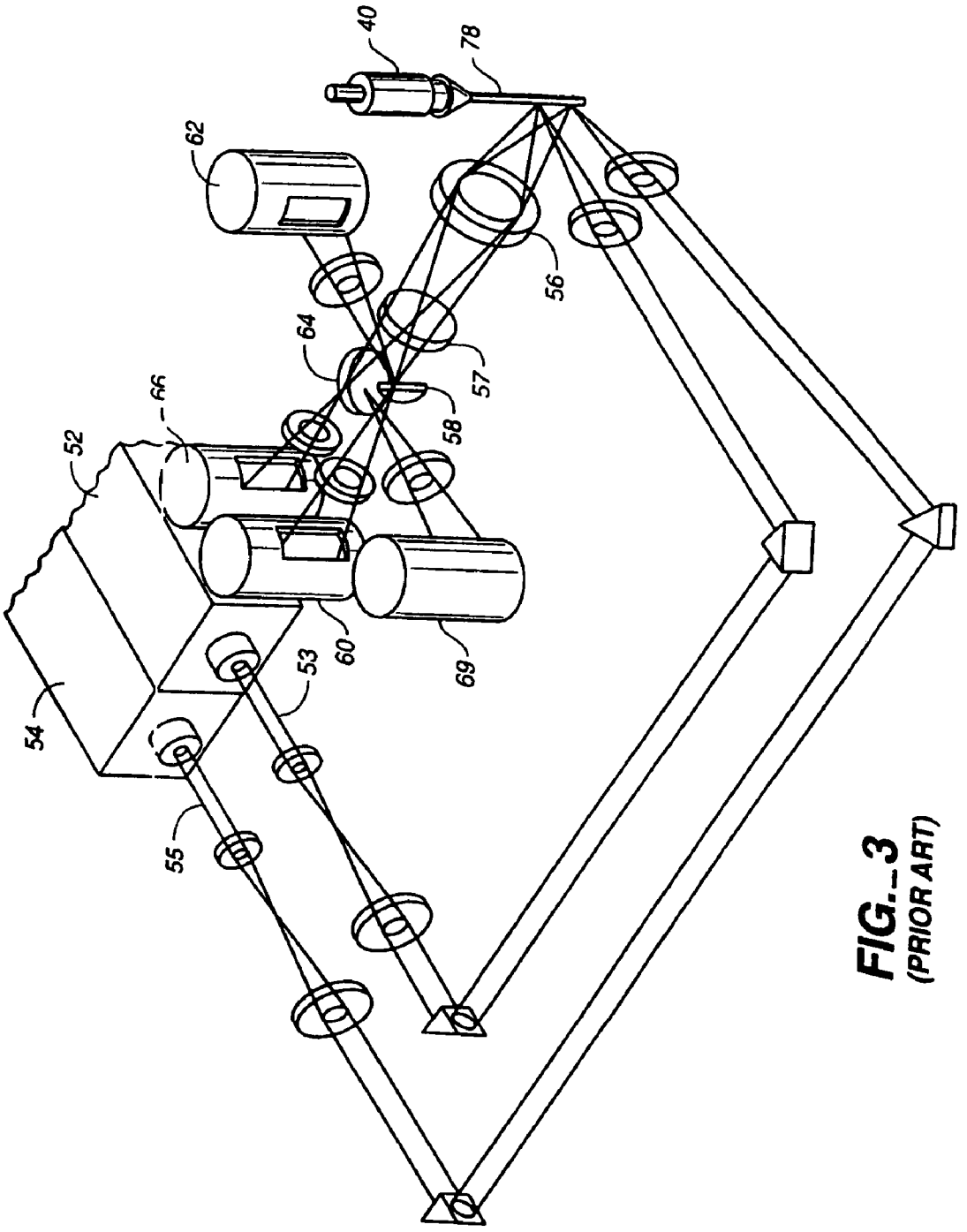
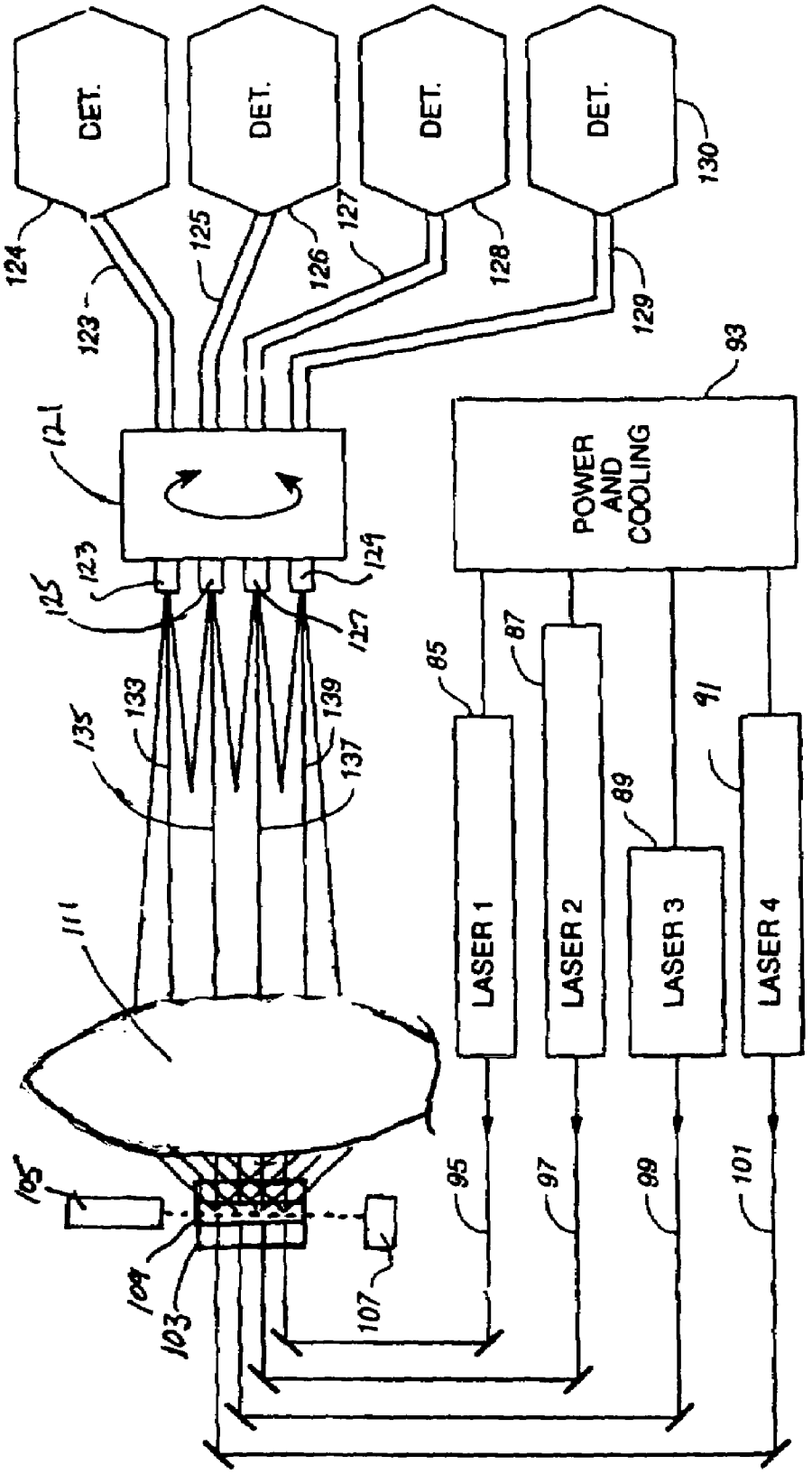


FIG._3
(PRIOR ART)


DET.
DET.
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POWER AND COOLING
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133
135
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LASER 1
LASER 2
LASER 3
LASER 4
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99
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FIG._4

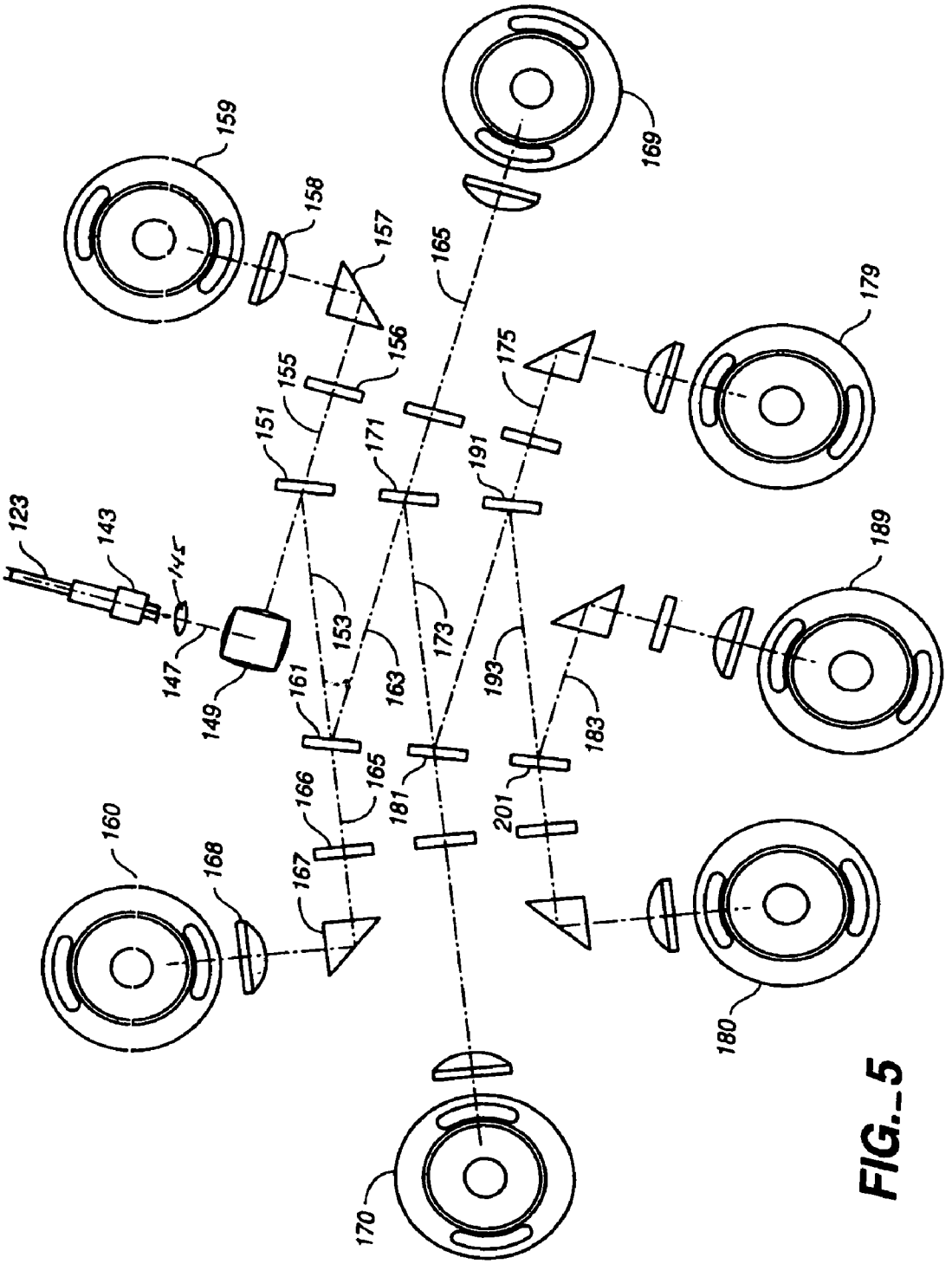
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FIG._5

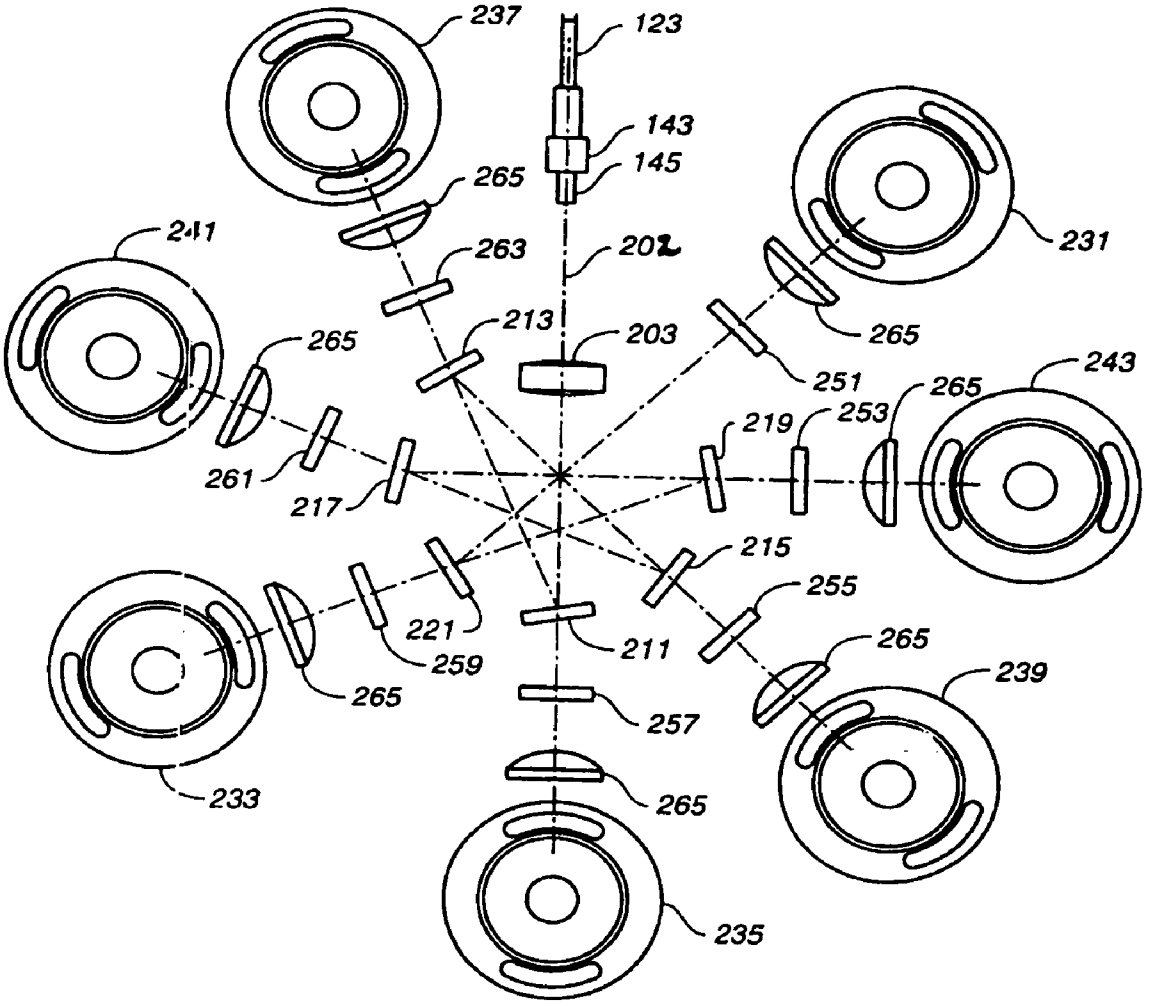
237
123
143
145
265
263
201
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FIG._6

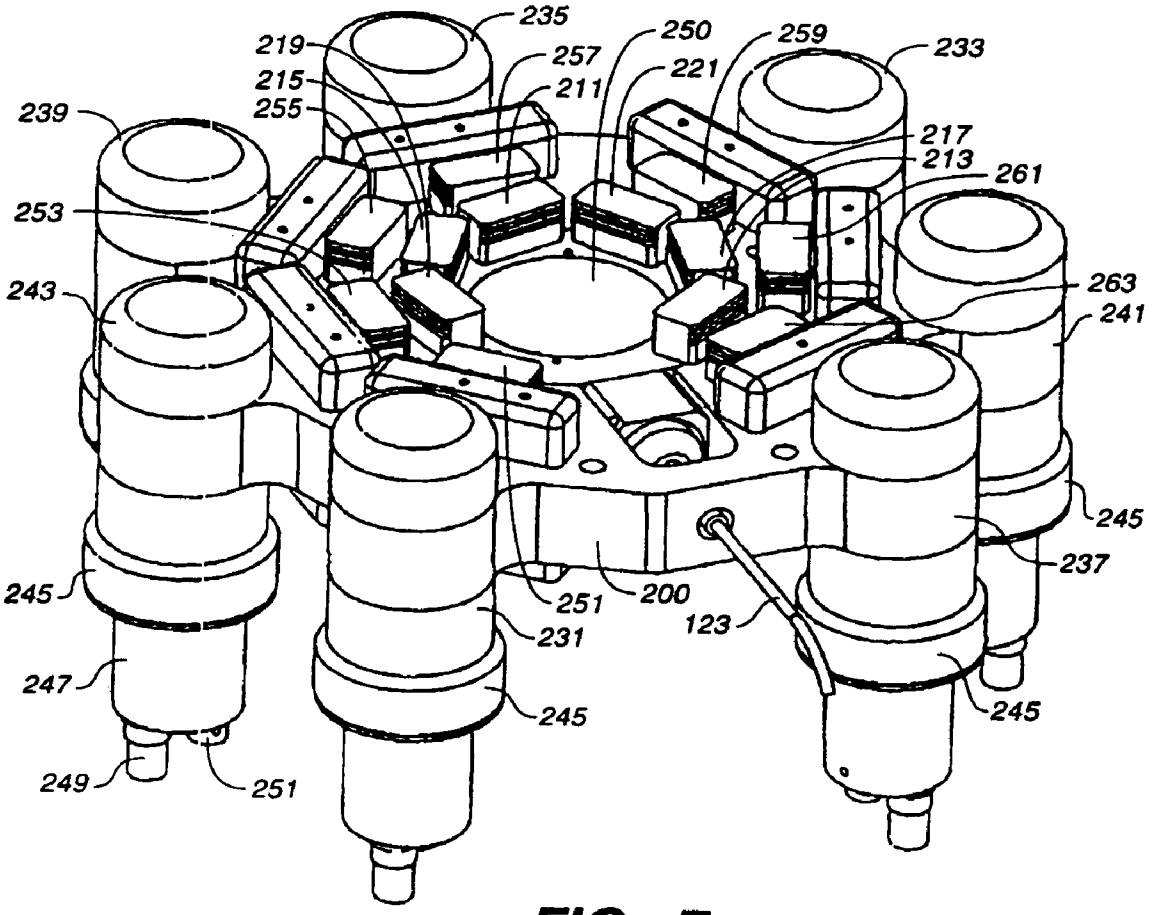
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FIG._7

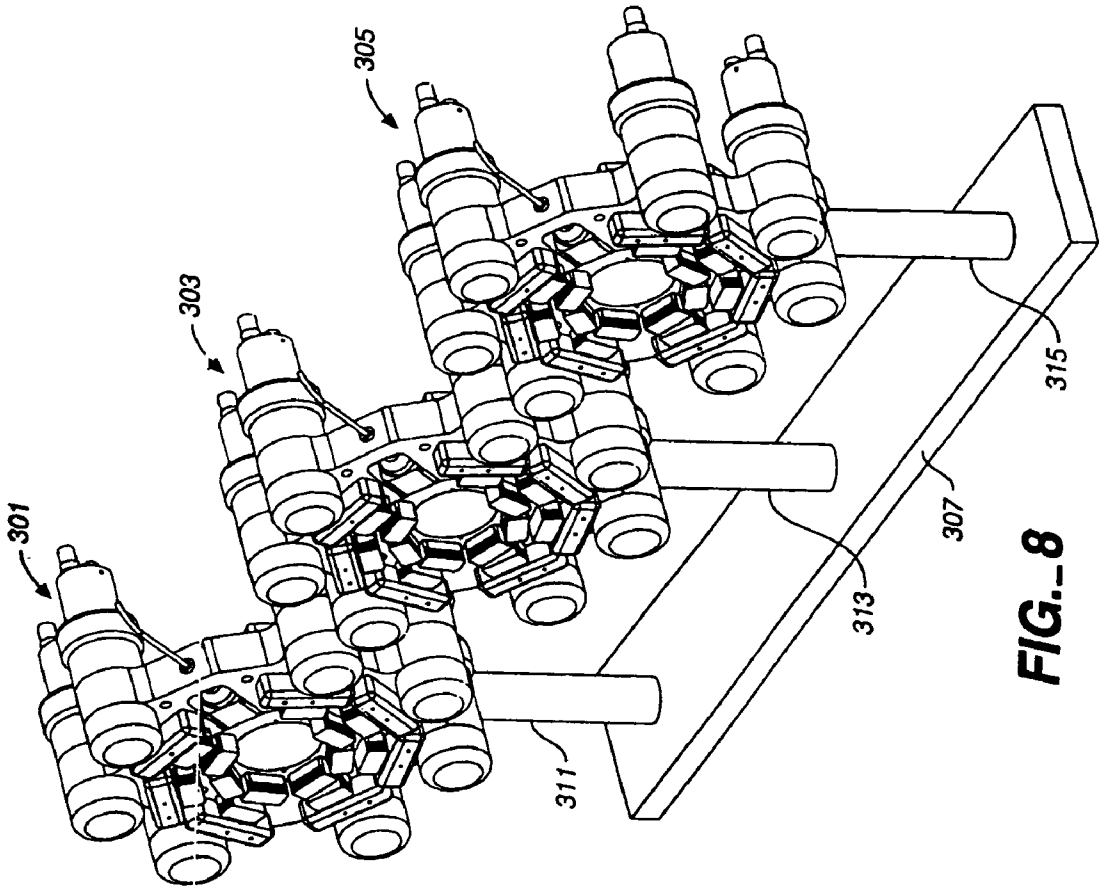

FIG._8

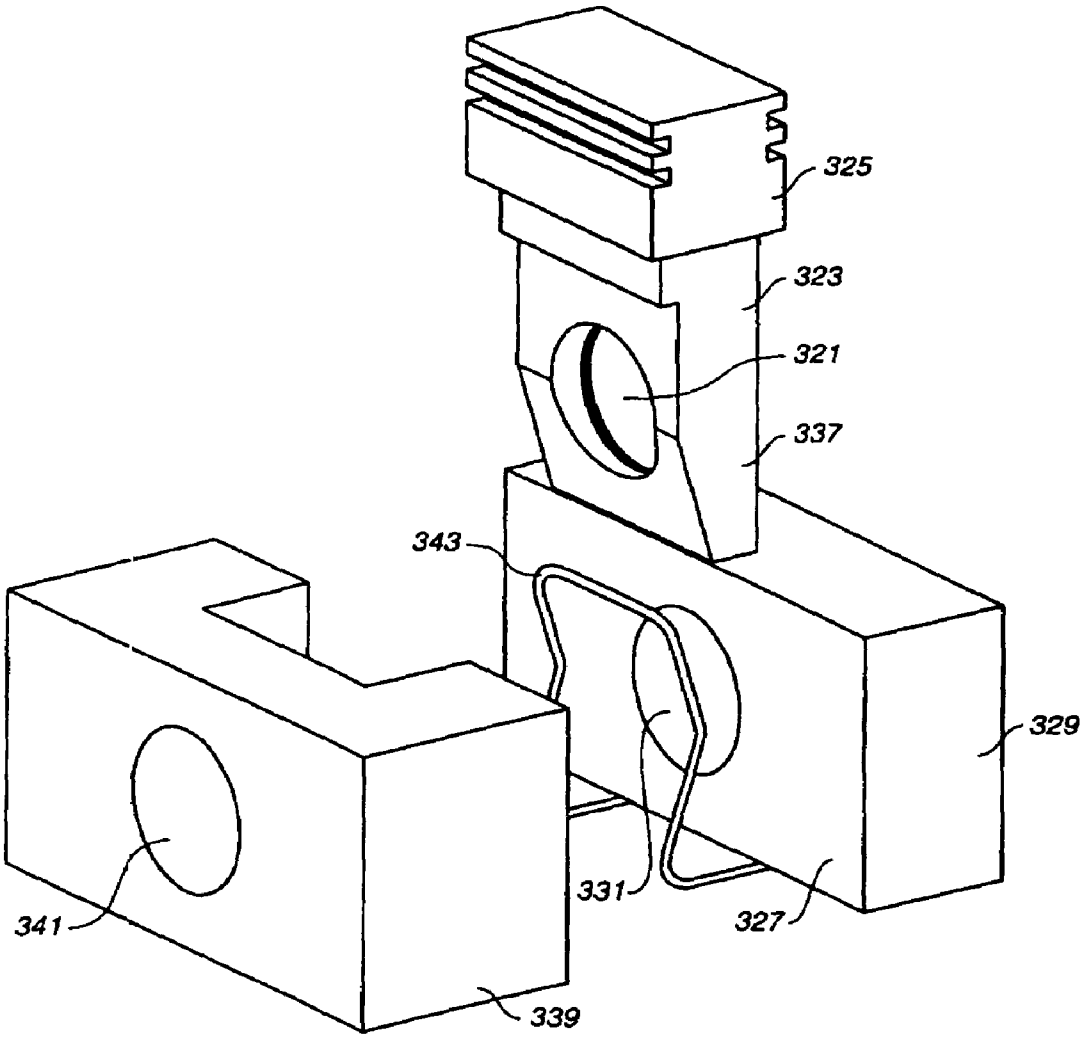

FIG._9

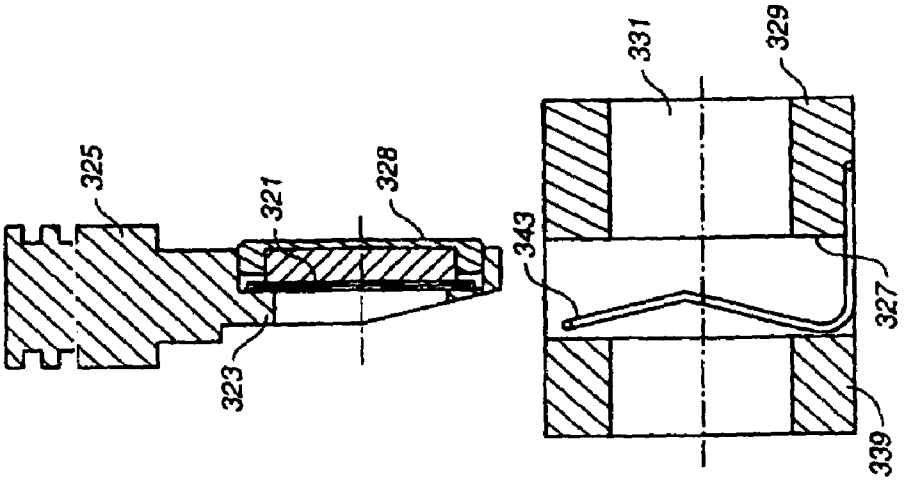

FIG._11

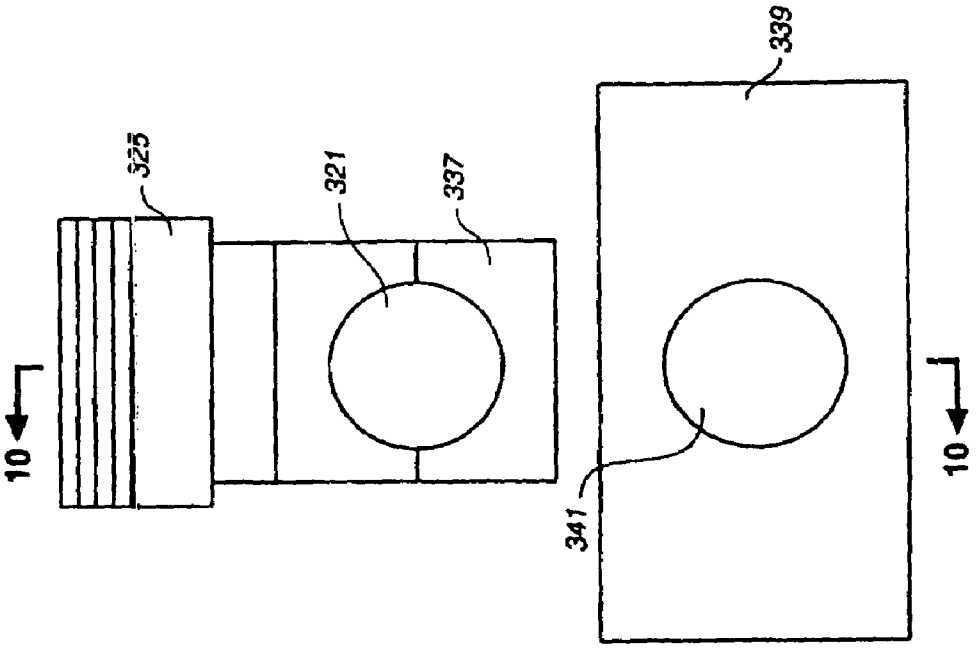

FIG._10

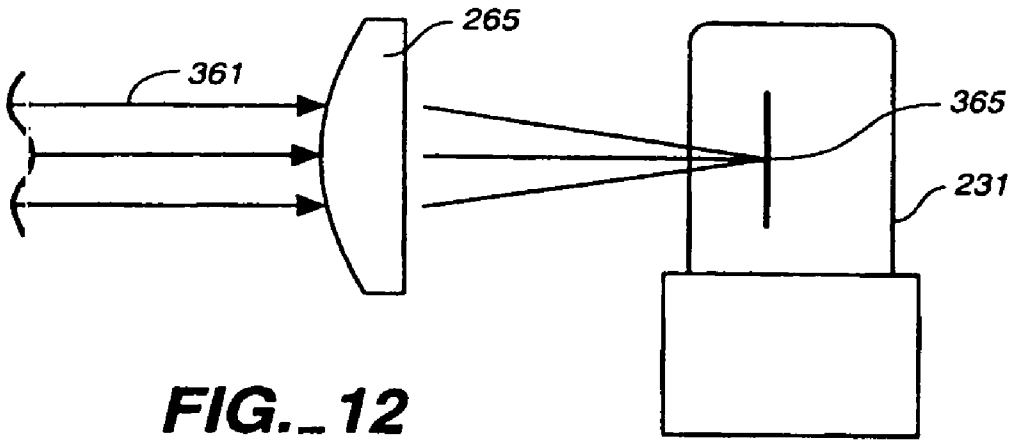

FIG._12

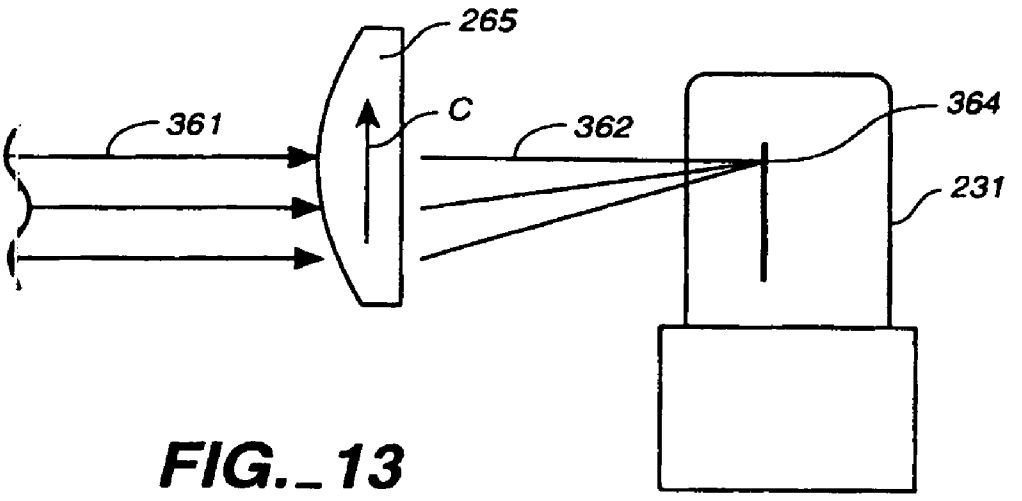

FIG._13

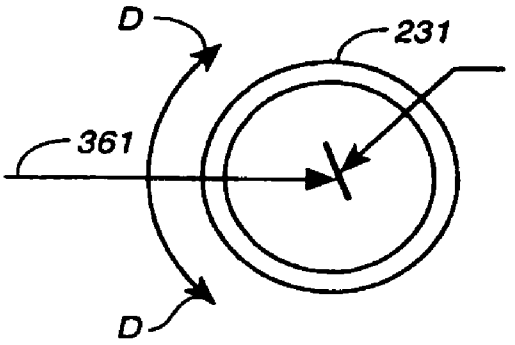

FIG._14

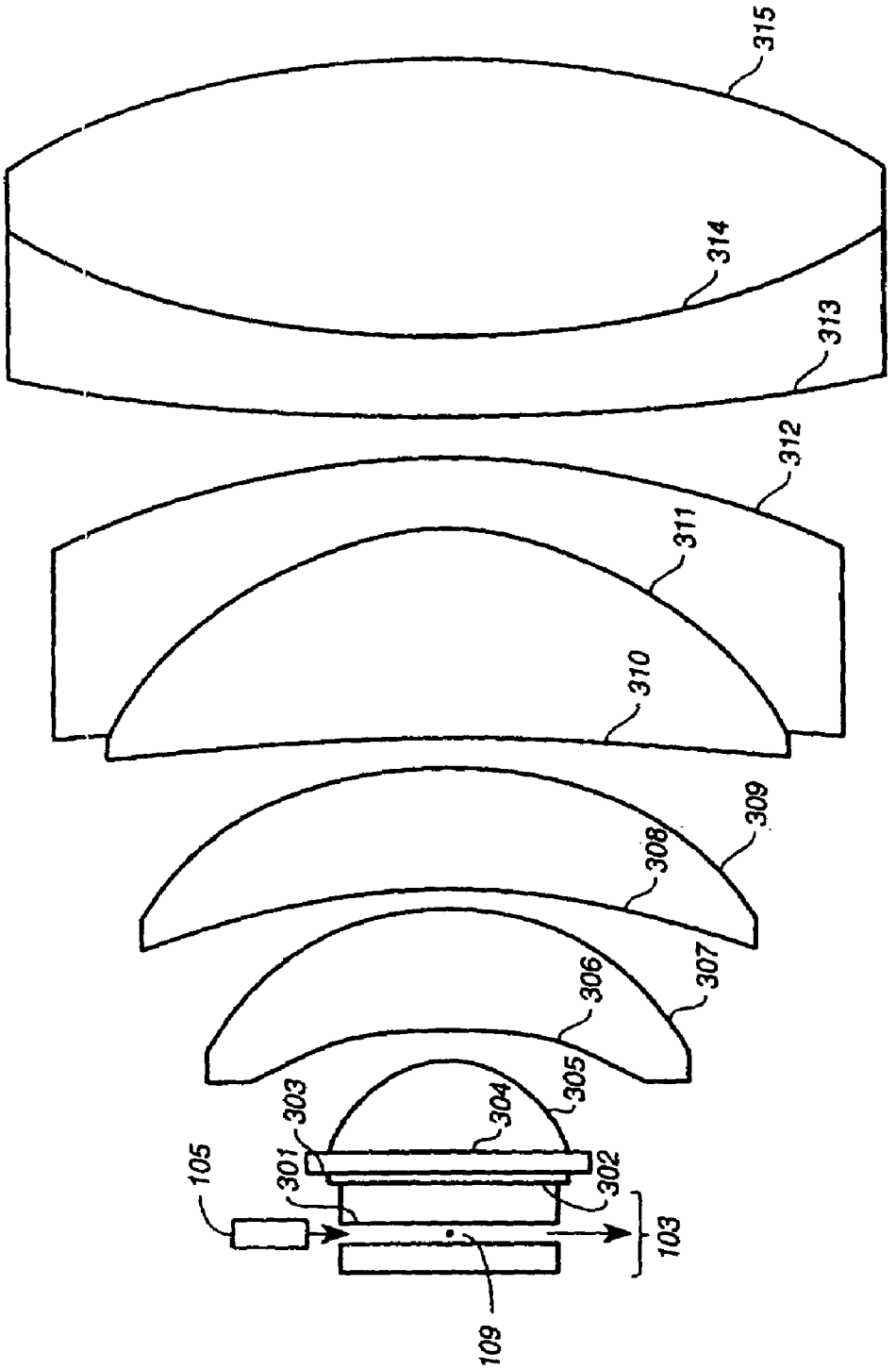
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FIG._15

FLUORESCENCE DETECTION INSTRUMENT WITH REFLECTIVE TRANSFER LEGS FOR COLOR DECIMATION

The present application is a continuation of U.S. Ser. No. 09/941,357, filed Aug. 28, 2001, which issued on Jan. 27, 2004, as U.S. Pat. No. 6,683,314.

TECHNICAL FIELD

The invention relates to analytical instruments for flourescent light analysis from target specimens and, more particularly, to such an instrument employing increased color decomposition of fluorescent signals from target substances.

BACKGROUND ART

As an example of fluorescent light decomposition for bioanalytical studies, in high throughput screening, the ability to simultaneously detect a plurality of fluorescent dyes with good wavelength discrimination enables deeper multiplexing and higher throughput. In another example using fluorescent light analysis, simultaneous detection of multiple dyes associated with cells allows simultaneous assay of cell surface antigens, organelle states, nucleic acid assay, and intercellular protein content to be detected in a single assay. Multiple wavelength detection requires detectors which can separate many bands of colors. This has commonly been done using dichroic mirror beam splitters.

U.S. Pat. No. 5,317,162 to B. Pinsky and R. Hoffman, assigned to the assignee of the present invention, describes an instrument for phase resolved fluorescent analysis. The architecture of that instrument is similar to prior art instruments which rely upon color decomposition of a beam of fluorescent light derived from a laser impinging upon a fluorescent target. Such an apparatus is described in the book *Practical Flow Cytometry,* by H. M. Shapiro, Third Edition (1995), p. 9. The book describes an apparatus similar to what is shown in FIG. **1**. A laser beam **12** from an air cooled argon ion laser **11** is used to generate a fluorescent signal which is subsequently decomposed or decimated. The beam **12** passes through focusing elements **13**, **14** and **15** to impinge upon a fluorescent substance in a flow cell **41**. Fluorescent target material, such as fluorescently tagged cells or particles within a liquid stream **16** flow through the flow cell **41**. Particles **43** having passed through flow cell **41** are collected in container **22**. Flow is adjusted by a fluidic system **18** which provides a hydrodynamically focused flow of cells within a sheath fluid. As the target substance passes through the flow cell, the focused light beam **12** intersects the liquid stream, causing fluorescent excitation, including the scattering of light. A photodiode detector **21** is positioned to receive forwardly scattered light. The fluorescent light is typically collected at an angle which is 90° relative to the excitation access of the light beam **12**. Axis **24** represents the 90° viewing axis for collection of fluorescent light. Objective lens **19** is placed across axis **24** to collect and collimate the fluorescent signal from the target substance. Fluorescent light collected by the lens **19** is formed into a beam **28** which impinges upon the dichroic mirror **25**. The dichroic mirror reflects light above 640 nm and transmits the remainder as the transmitted leg **30**. Reflected leg **31** is directed to the red light fluorescence detector photo multiplier tube (PMT) having a 660 nm longpass filter. Detector **32** thus registers the red light component of the collected fluorescent signal from the flow cell **41**. The transmitted leg **30** impinges upon

the dichroic mirror **34** which reflects light above 600 nm. The reflected leg **35** is orange light which is detected by the orange fluorescence detector PMT **33** having a 620 nm bandpass filter. The transmitted leg **36** impinges upon the dichroic mirror **38** which reflects light above 550 nm and transmits the remainder in transmitted leg **42**. Reflected leg **39** is detected by the yellow fluorescence detector PMT **40** having a 575 nm bandpass filter.

The transmitted leg **42** impinges upon dichroic mirror **44** which reflects light above 500 nm. The reflected leg **46** impinges upon the green fluorescence detector PMT **47**, while the transmitted leg **48** consists of essentially blue light which is directed into the orthogonal scatter detector PMT **50** with a 488 nm bandpass filter, registering blue light. In this manner, the fluorescent signal in beam **28** collected by collector lens **19** is decomposed into five colors with the amplitude of each detector being recorded simultaneously to form a spectral characteristic of the fluorescent material illuminated by the laser beam.

The flow cell **41** is typically a flat-sided quartz cuvet of square or rectangular cross-section with a flow path therethrough. Such a quartz cuvet of the prior art is described in international patent publication WO 01/27590 A2, owned by the assignee of the present invention, shown in FIG. **2**.

In that international patent application, the flow cell mentioned above is described with an aspheric reflective light collector, unlike the lens **19** shown in FIG. **1**. The apparatus of the international patent application mentioned above is shown in FIG. **2** where a flow cell **17** is a quartz block having a flow channel **20** where a liquid stream containing fluorescent material is directed through the cell in a stream controlled by a nozzle. The flow cell of FIG. **2** has a reflective aspheric light collector **51** collecting light scattered to a side of the flow cell opposite the side where lens **19** is situated. An aspheric reflective element **51**, placed on the side of flow cell **17** opposite collector **19** serves to augment the light directed toward lens **19**, or in some cases performs the function of lens **19**. The reflective collector **51** is coated with a broadband reflecting material for augmenting the amount of light collected from the flow cell. The aspheric shape may be parabolic or ellipsoidal, having focal properties to match light collector **19** of FIG. **1**.

The apparatus of FIG. **3** is described in U.S. Pat. No. 4,727,020 to D. Recktenwald and assigned to the assignee of the present invention. This device shows a pair of lasers **52** and **54** directing light to a flow cell **78** so that two different illumination profiles may be used to illuminate a sample. Each laser is selected for stimulating the desired fluorescent emission from target substances. A set of detectors is associated with a different color band. For example, laser **52** generates a beam **53** impinging upon the flow stream **78** and producing a fluorescent signal collected by lens **56**, focused by lens **57** onto dichroic mirror **58**, a beam splitter, for analysis by detectors **60** and **62**. Similarly, laser **54** generates a beam **55** which impinges upon the flow which includes the particles under study in air and generates scattered fluorescent light, collected by light collector **56** and imaged by lens **57** onto dichroic mirror **64** where the beam is split between detectors **66** and **69**. In summary, it is known that groups of detectors can be associated with different lasers simultaneously illuminating the same target substance.

An object of the invention was to provide an improved system for detecting fluorescent light having multiple colors emitted from a target using a greater number of detectors than has been achieved in the prior art.

SUMMARY OF THE INVENTION

The above object has been achieved in an optical instrument having a detector arrangement featuring a larger number of spectrally diverse detectors than previously available. The detectors are fed by a plurality of lasers of different colors with parallel, spaced apart beams impinging upon fluorescent target material at different locations which may be in a channel, a plate, or the like. By using a plurality of lasers, a wide range of spectral responses may be stimulated from fluorescent target material. The target material may be fixed or flowing. Spatially separated fluorescence associated with each beam and emanating from the target material is collected by a large numerical aperture collector lens that preserves the spatial separation of the light originating from the plurality of sources, i.e. the fluorescent signatures of the laser beams on the target material is preserved. Fluorescent light stimulated by the different sources is imaged into a plurality of optical fibers that carry the light to separate detector arrays. Each array has a series of beam splitters in a series or cascade arrangement receiving light from an associated fiber and relaying part of the light to a downstream beam splitter, spectrally filtering the light on each relay within the cascade arrangement by means of coatings associated with the splitters. Within each array, light reflected from a beam splitter is forwarded to a downstream splitter, while light transmitted through a beam splitter is sent to a detector. This means that the reflected component is a broadband wavelength component and the transmitted component is filtered to be a narrowband wavelength component. For the last beam splitter, light from the reflective leg may be sent to a detector, as well as light from the transmitted leg. Since, for most optical coatings on a beam splitter, the fraction of light reflected from a beam splitter exceeds the transmitted fraction, the downstream beam splitters receive more light from the reflective transfer legs than the prior art arrangement where downstream beam splitters receive light from the transmitted transfer leg. Each array of detectors is arranged in a polygonal compact cluster. The detector configuration of the present invention is modular because light from each laser is spatially separated from other lasers and each detector cluster has at least 6 detectors. The clusters may be physically separated since optical fibers can feed light to clusters in remote locations or in stacks or racks. In this instrument, collected light is transmitted to a plurality of beam splitters. Note that the beam splitters, split light into a transfer leg and a transmitted detector leg, as in the prior art. However, unlike the prior art, the transfer leg is reflected from beam splitters and forwarded to another beam splitter and the transmitted detector leg is directed to a detector. This is true for a majority of beam splitters, but not for the last one receiving a maximally attenuated transfer leg where the transfer leg is either sent to a detector or terminated. So the last dichroic mirror may be associated with two detectors, one for the reflected leg and one for the transmitted leg. By using reflective transfer legs for most detectors, the detectors may be clustered in a polygonal arrangement of between five and ten light detectors in a common plane. Here, the term "most detectors" refers to all transfer legs except the last one, but is not limited to the last leg.

By maintaining spatial separation for the input beams, spatial separation can be preserved in the output transfer beams, with each transfer beam directed into an optical fiber for delivery to a detector cluster. This allows detector clusters to be stacked or placed in racks, with optical fibers carrying transfer beams to the location of an input port of each cluster. Once inside of a cluster, the transfer beam is decimated by the dichroic beam splitters, each beam splitter inclined to a transfer leg at a preferred angle centered on 11.25 degrees. Other angles will work but not as efficiently. Each beam splitter achieves color separation in the usual way, i.e. by transmitting light of a particular wavelength. This transmitted light is directed to a photomultiplier tube, or the like, which is positioned, to the extent possible, to detect light in the transmitted detector legs associated with the split beam. A focusing lens and the detector photomultiplier tubes are positionally relatively adjustable so that an optimum detector position can be found by motion of a detector element relative to a lens focusing incoming light. In this manner, the fluorescence associated with each of several laser beams is simultaneously decimated into bands characteristic of the target material within the detector array of each cluster. A group of clusters provides color decimation much greater than heretofore available. Moreover, the apparatus is modular because a greater number of fibers can feed a greater number of clusters. One of the advantages of using a reflective transfer leg to relay the optical signal for decimation, rather than the transmitted leg, is that the reflective transfer leg is a stronger optical signal. After encounters with several beam splitters, the signal attenuation in a relayed reflective transfer leg signal is substantially greater than for an optical signal in which the relay was transmitted through an equal number of beam splitters, as in the prior art.

In one embodiment, the light collection and detection optics are included in a system having a plurality of lasers producing input beams of different wavelength profiles to simultaneously illuminate a fluorescent target, usually fluorescent particulate matter which could be discrete small particles, including cells, or large biological molecules. The term "color decimation" refers to the simultaneous spectral breakdown of polychromatic light beams from a target substance into narrow bands of light arriving at detectors. Scattered light is measured by other detectors not relevant to this invention or this application. Scatter detectors are not described herein. Collection of fluorescent light is by a lens similar to a microscope immersion lens of large numerical aperture, the lens forming output transfer beams directed to a plurality of dichroic mirrors. After collection, the light is imaged into fibers, then distributed to "n" clusters of "m" detectors, yielding an "n" times "m" number of detectors resolving the fluorescent light stimulated by the input beams. Each cluster isolates light within the corresponding array of detectors. Clusters may be mounted on rails, racks or stacks in a modular arrangement.

DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective plan view of a multi-color flow cytometer with single wavelength excitation in accordance with the prior art.

FIG. **2** is a perspective view of a light collector of the prior art for use with a flow cytometer of the kind illustrated in FIG. **1**.

FIG. **3** is a perspective view of a multi-color flow cytometer with plural wavelength excitation and a polygonal arrangement of detectors in accordance with the prior art.

FIG. **4** is a plan view of a multi-color optical instrument of the present invention.

FIG. **5** is a top plan of a planar polygonal detector arrangement showing decimation of an incoming beam with

reflective transfer leg beam splitters in accordance with the present invention, the incoming beam received from a fiber bundle illustrated in FIG. **4**.

FIG. **6** is a top plan of an alternate planar polygonal detector arrangement showing decimation of an incoming beam with reflective transfer leg beam splitters, as in FIG. **5** but with the beam having a folded path for compact placement of the detectors.

FIG. **7** is a perspective assembly view of the apparatus of FIG. **6**.

FIG. **8** is a perspective plan view of three detector arrays of the kind shown in FIG. **7** mounted on a rack in accordance with the present invention.

FIG. **9** is a perspective plan view of a detail of a mirror holder used in the apparatus of FIG. **7**.

FIG. **10** is a front elevation of the mirror holder illustrated in FIG. **9**.

FIG. **11** is a side sectional view of the mirror holder of FIG. **10**, taken along lines **10**—**10**.

FIG. **12** is a side plan view of a detector photomultiplier tube and optics used in the detector arrangements shown in FIGS. **5** and **6**.

FIG. **13** is a side plan view of a motion to optimize the sensitivity of the detector shown in FIG. **12**.

FIG. **14** is a top plan view of a motion to optimize the sensitivity of the detector shown in FIG. **13**.

FIG. **15** is a plan view of a light collector lens for use in the instrument input end arrangement illustrated in FIG. **4**.

BEST MODE FOR CARRYING OUT THE INVENTION

With reference to FIG. **4**, a first laser **85**, a second laser **87**, a third laser **89**, and a fourth laser **91**, all produce light with unique wavelength profiles and all are connected to respective power supplies and a cooling module **93**. The lasers emit respective beams **95**, **97**, **99** and **101** which are directed by means of beam-turning mirrors toward flow stream **103** causing the beams to intersect with the stream. Although the preferred embodiment features a flow cytometer, this instrument is merely illustrative of instruments which employ fluorescence detection and color separation. Other instruments include microscopes, electrophoresis instruments, spectrophotometers, and the like. The scope of the present invention is therefore not limited to flow cytometers.

A fluidic system **105** feeds tagged target liquid substances into a stream **103** in a controlled manner. Material which passes through the beam illuminated zone is collected in collection cup **107**. The illuminated zone is established by the four laser beams that impinge upon fluid tagged target material, thereby causing scattering and fluorescence. Each beam has a characteristic color produced by different types of lasing material. For example, characteristic laser illumination wavelength profiles may be produced by CO_2 lasers, argon ion lasers, copper vapor lasers, and helium neon lasers. Other colors are available from different types of lasers. The output power of each laser is typically between 10 and 90 milliwatts. At such power levels, a sufficiently strong optical signal is produced without damaging coatings on the surfaces of mirrors, fibers or beam splitters. Coatings are selected to achieve desired passband transmissions and may be specified from coating manufacturers.

Light of different colors intersects the flow stream **103** and interacts with fluid sample causing scattering and fluorescence that is spatially separated along a line parallel to the flow channel **109**. Scattered light can be processed by well-known scatter detectors. For simplicity, this description deals only with fluorescent light. This light appears to be originating at four spaced apart point sources or spots, each of which is imaged by a lens light collector **111** to four respective optical fibers **123**, **125**, **127** and **129**, all held in place by a movable holder **121** which securely mounts the fibers and allows both rotational and axial adjustment of the fibers relative to light collector **111**. In other words, the holder **121** may be moved so that the fibers optimize the input light into the fibers from collector **111**. The focal spots produced by collector **111** enter the tip of each respective fiber **123**, **125**, **127** and **129**, each of which is a multi-mode fiber.

Light collector **111** is a group of lens elements which is described with reference to FIG. **15**. The collector is placed very close to the flow stream, within a few millimeters. The distances shown in FIG. **4** are not to any scale and are out of proportion. This collector gathers fluorescent light from the fluorescent target material. The collector lens is a microscope objective lens similar to the fluid emersion microscope objective lens shown in U.S. Pat. No. 5,805,346, except that the lens of the present invention is a positive meniscus lens, while the fluid emersion microscope objective lens of the '346 patent is a negative meniscus lens. Other differences exists, but the lenses are similar in the number of optical elements and their arrangements. Various supports may be used with the goal of reducing vibration and allowing proper alignment of optical elements along the optical axes **133**, **135**, **137**, and **139** defined by collector **111**. The optical axes are maintained by each of the fibers, although each of the fibers may be bent to remove light to the location of a detector array. Light in each of the fibers **123**, **125**, **127** and **129** is transmitted to a respective detector cluster **124**, **126**, **128** and **130** which houses an array of detectors. Each array processes fluorescent light which has maintained spatial separation, i.e. color independence, to a large extent. In other words, the fluorescence stimulated by a particular laser has been preserved and forwarded to an array of detectors which operates independently from other arrays of detectors. Each array of detectors differentially separates bands of light by filtering, using coatings on beam splitters and lenses, in a known manner. With each cluster having between 3–4 and 10 or more detectors, each detector receiving a passband of between 10 and 75 nanometers, the instrument of the present invention has a wide spectral response.

Details of a detector array or cluster are shown in FIG. **5**. The optical fiber **123** has a fiber terminal **143**, which allow emergence of light and formation of a beam by means of a collimating lens **145**. The output beam **147** is directed toward a beam-turning mirror **149** directing light at a first beam splitting element **151** which is a dichroic mirror transmitting light in beam **155** in a transmitted detector leg toward a filter **156**, a beam-turning element **157**, and a focusing element **158** which directs the beam onto a light sensitive element of a photomultiplier tube **159**. The tube is adjusted so that its most sensitive area is exposed to the incoming beam. This beam is known as the transmitted detector leg because it is transmitted through the beam splitting element **151**. The beam splitting element has a coating which allows transmission of one band of optical signals, while reflecting light in another band in the form of beam **153** which forms a reflective transfer leg for the light which was originally in beam **147**. The reflective transfer leg **153** is seen to fall upon another beam splitting element **161** which is a dichroic mirror having different optical characteristics from the beam splitter **151**. The colors removed by beam splitter **161**, as well as the other beam splitters, are different, each beam splitter removing a selected band of

colored light in the same manner, but different wavelengths, as in beam splitters of the prior art described with reference to FIG. **1**.

Beam splitter **161** has a reflective transfer leg **163** reflected from the surface of the splitter, as well as a transmitted detector leg **165** transmitted through the beam splitter to the filtering element **166**, the beam-turning element **167** and the focusing element **168**. The transmitted beam impinges upon a sensitive portion of detector **160** where the amount of light associated with the band transmitted by beam splitter **161** is measured. In the same manner, beam splitters **171**, **181**, **191** and **201** split incoming beams which are light beams reflected from upstream beam splitters, with beam splitter **191** being upstream of beam splitter **201**, beam splitter **181** being upstream from beam splitter **191**, and beam splitter **171** being upstream from beam splitter **181**, etc. Each beam splitter, except for the last one, beam splitter **201**, separates light into a reflective transfer beam, with the transfer beam **173** being reflected from beam splitting dichroic mirror **171** and the transmitted beam through dichroic mirror **171** being beam **165** impinging upon detector **169**. On the other hand, the reflected beam **173** is transmitted to beam splitter **181**, with the transmitted leg being directed to detector **170**. Beam **175**, passing through the beam splitter **191**, is directed to a detector **179** while the reflected leg **193** goes to the last beam splitter, namely dichroic mirror **201**. This element, unlike the other beam splitters, has two detectors associated with it. One detector **180**, receives light transmitted through the beam splitter **201** toward detector **180** while light reflected from beam splitter **201** forms a reflected leg **183** which impinges upon detector **189**. In this manner, all of the detectors illustrated in FIG. **5** form an array which decimates light from a single fiber **123**. As mentioned previously, the fiber **123** is associated with scattered and reflected light collected from one of the lasers mounted on the optical bench. Thus, for each laser there is an array of detectors in a cluster. In FIG. **5**, the transfer leg forwarded upstream from one beam splitter to the next follows a zigzag pattern. In FIG. **6**, the transfer legs intersect in a star-shaped pattern yielding a more compact polygonal arrangement of detectors.

In FIG. **6**, the optical fiber **123** is terminated in a terminal **143** directing an output beam **202** through a collimating lens **203** and thence onto an array of beam splitters **211**, thence to beam splitter **213**, then to beam splitter **215**, then to beam splitter **217**, then to beam splitter **219**, and, lastly, to beam splitter **221**. The arrangement of beam splitters is in a polygonal pattern. In each case, the transfer leg is reflective, with the beam splitter being a dichroic mirror which is inclined at an angle of 11.25° to perpendicular, i.e. a small angle, say between 5° and 20° . It has been found that this angle optimizes balance between reflection and transmission. On each bounce from a beam splitter, part of the beam called the "detector leg" is transmitted through the beam splitter toward one of the detectors. Detector **231** is associated with the transfer leg of beam splitter **221** while the detector **233** is associated with the detector leg from the same beam splitter. Detector **235** is associated with the detector leg coming through beam splitter **211** while detector **237** is associated with the detector leg through beam splitter **213**. Detector **239** is associated with the detector leg through beam splitter **215** while detector **241** is associated with the transfer leg from beam splitter **215** and the detector leg through beam splitter **217**. Correspondingly, the detector **243** is associated with the transfer leg from beam splitter **217** and the detector leg through beam splitter **219**. Each of the beam splitters is a dichroic mirror having different wavelength characteristics for decimating the input beam **202** into different colors which register at the different detectors. Coatings applied to the dichroic mirrors account for reflection of some wavelengths and transmission of other wavelengths. Laser light of a particular frequency will stimulate fluorescent emission in generally known wavelength bands. Light in these bands is collected and passed through an optical fiber to a detector array, the detectors arranged in a polygonal pattern of greater circumference than the polygonal pattern of beam splitters. Within each cluster decimation of the light occurs, with passbands of 10–75 nanometers registering at each detector, depending on the sharpness of filtering of the coatings applied to the beam splitters. Additional selectivity of the signals reaching the detector may be gained by a series of filters **251**, **253**, **255**, **257**, **259**, **261** and **263**, the filters arranged in a polygonal pattern with a polygonal circumference greater than the circumference of the beam splitters but less than the circumference of the detectors. Each of these filters is placed in front of a corresponding detector. The filter has a bandpass over a range of wavelengths which is of particular interest in the corresponding detector. Associated with each detector is a focusing lens **265** for focusing light in a detector leg on a sensitive spot of the detector. Each lens **265** is movable for adjusting the focal spot during calibration of the instrument.

In FIG. **7**, a cluster with an array of detectors and an array of beam splitters is seen to be held in place by a frame **200** which generally supports detectors **237**, **241**, **233**, **235**, **239**, **243** and **231** in a polygonal array where the polygon is drawn connecting the axial centers of each of the cylindrical detectors, the detectors being photomultiplier tubes. Each tube is seated in a tube mount **245**. A tube connector base **247** makes contact with pins of each tube. An electrical feed-through **249** allows power to come to connector base **247** while signals exit the tube through another feed-through **251**. Similar connector bases and feed-throughs exists for each tube. Within the center of the frame **200** is a coverplate **250**. About a first close distance from the coverplate is a polygonal array of dichroic mirror holders for the dichroic mirrors **213**, **217**, **221**, **211**, **215** and **219**. A slightly further distance are the filter holders **263**, **261**, **259**, **257**, **255**, **253** and **251**. The dichroic mirror holders and the filter holders are mounted in vertically removable housings so that dichroic mirrors and associated filters may be interchanged or replaced.

In FIG. **8**, a plurality of clusters **301**, **303** and **305** is shown to be vertically mounted on a rack **307** by means of standoff supports **311**, **313** and **315**. The standoff supports are merely illustrative of the manner in which three arrays may be mounted on a rail or rack for easy replacement or modular supplementation. Each cluster is of the type shown in FIG. **7**.

In FIGS. **9**–**11**, the construction of a removable beam splitter holder is shown. The beam splitter mirror **321** is held in a mirror holder frame **323** at a desired angle. Frame **323** is supported by a block **325** having channels for finger contact in a non-slip manner. The mirror holder frame **323** has a flat side **328** which presses against the flat side **327** of a seating block **329** having a central aperture **331** corresponding to the position of mirror **321**. A facing block **339** has an aperture **341** in alignment with aperture **331** and with mirror **321**. The flat side **327** is a reference surface for positioning of the mirror **321**. A wire spring **343** serves to push the mirror holder frame **323** against the block **329**.

FIGS. **12**–**14** show how the transmitted leg of a beam **361** may impinge upon the focusing lens **265**. With motion of the focusing lens **265**, as shown in FIG. **13** in the direction of the

arrow C, the focused beam **362** is moved to a more sensitive spot **364** on the photomultiplier tube **231** in comparison to a less sensitive location **365** shown in FIG. **12**. Additional sensitivity may be gained by slightly rotating the photomultiplier tube **231** with its housing, within the support frame to optimize the output signal for a particular detector leg **361** focused on a detection element in the photomultiplier tube. Motion is indicated by the arrows D.

FIG. **15** shows the construction of light collector **111** in FIG. **2**. A flow cell **103** is shown at the left of the drawing with flow channel **109** and input fluid from fluidic system **105** passing through channel **109**. As has been previously noted, a flow system is but one type of optical instrument where fluorescence can be observed. Non-flow systems may also be employed with the detection apparatus of the present invention. A large numerical aperture ("N.A.") lens system, i.e. N.A. greater than one, positioned as shown in FIG. **4** is described according to the lens data contained in the following table. The numbered optical surfaces in the figure correspond to surface numbers in the leftmost column of the table. All radii and thickness values are in millimeters. Surface curvature tolerances for the lens data include 5 fringes for power (deviation of actual curvature from nominal curvature) and 1 fringe for irregularity (deviation from a perfect spherical surface). Tilt tolerance is 0.05 degrees from normal in any direction. Material tolerances are 0.0005 for refractive index and 0.8% for Abbe number.

Surface	Radius of Curvature	Thickness	Thickness Tolerance	Aperture Radius	Clear Aperture Radius	Material
109	4	0.0889	—	0.2		Water
301	4	1.94	—	4.6		Silica
302	4	0.1682	—	4.6		Gel
303	4	0.8	.025	5.1	4.6	BK7
304	4	3.915	.025	4.6	4.6	BK7
305	−4.66	1.5	.025	4.6A	4.6	Air
306	−16.918	5	.05	8.5	7.3	BK7
307	−10.894	1	.025	10	8.8	Air
308	−26.836	5	.10	11.5	10.2	BK7
309	−15.008	1	.025	12.5	11.1	Air
310	−103.704	9	.10	13	11.9	BK7
311	−14.012	3	.10	13	12.2	SF8
312	−34.38	2	.05	15.5	14.1	Air
313	+123.446	3	.10	17	14.9	SF8
314	+34.38	12	.10	17	15.1	BK7
315	−36.554	126.731	.50	17	15.5	Air

The lens proper (surfaces **303** through **315**) in this system is adapted to magnify and view cellular material within a cytometry flow cell or cuvette **103** (the flow cell inner and outer wall surfaces being optical surfaces **301** and **302** above). As indicated in the table, a flow cell has 0.007 inch (0.1778 mm) interior dimensions (wall-to-wall) and the fluorescent targets **109** to be detected and analyzed are immersed in saline water flowing through the cell **103**, nominally for lens design purposes through the center of the cell a distance of 0.0889 mm from the cell's inner wall. The 1.94 mm thick, fused silica, planar cell wall has a refractive index_{nD} of 1.45857 and an Abbe number_{vD} of 67.7. An optical gel layer provides an interface between the cytometry flow cell and the lens proper and improves lens mounting tolerances. The gel material is preferably NyoGel OC-431A sold by William F. Nye, Inc. of New Bedford, Mass., and has refractive indices at the 0.40 μm, 0.55 μm and 0.70 μm principal lens design wavelengths, respectively, of 1.487, 1.467, and 1.459. The gel should have a thickness less than 0.5 mm, and is selected in the above design to be 0.1682 mm thick. Other cytometry flow cells with different interior and wall dimensions, and other optical gels or oils could be used, with appropriate modifications in the lens specifications, optimized using commercially available software. Although lens positioning tolerances would be much tighter (0.025 mm or less), the lens could also be integrated with or mounted to the flow cell without using optical gel.

The lens glass types BK7 and SF8 (Schott glass designations) have been selected because they are relatively inexpensive stock materials that are easy to obtain in quantity, and because they are easy to grind and polish and don't stain easily. Other glass types could be used instead, including similar glass types from other optical glass suppliers, with appropriate modifications in the lens specifications. The optical glass designated BK7 [517642] has a refractive index_{nD} of 1.51680 and an Abbe number_{vD} of 64.17, and the optical glass designated SF8 [689312] has a refractive index_{nD} of 1.68893 and an Abbe number_{vD} of 31.18. All of the lenses in the preferred embodiment have spherical surfaces because they are inexpensive, more readily available in bulk, are more alignment tolerant, and are easier to assemble and test than aspheric lenses. However, if desired, modified lens specifications using one or more aspheric lenses have lower on-axis aberrations and could be used, although from a commercial standpoint the performance improvement likely would not be sufficient to justify their significantly greater expense and assembly difficulty.

The basic lens requirements include a numerical aperture of at least 1.17. (An object N.A. of 1.20±0.01 was used in obtaining the preferred embodiment that is set forth in the table above. A numerical aperture of 1.20 provides about 10 to 15% greater light collection than one of 1.17) The field of view should be at least 200 μm diameter and, if possible, as much as 400 μm or better. The present preferred embodiment has a field of view of 400 μm diameter. The working distance should be at least 1.75 mm, (2.2 mm is achieved in the preferred embodiment.) Most importantly, a lens system of less optical aberrations and high image quality is required for better resolution compared to existing cytometry lenses. In particular, the RMS spot size (a measure of resolution) in image space (for hypothetical point objects) for all wavelengths and all field points should be at most 100 μm. The present preferred embodiment achieves a calculated geometrical spot size of 85.04 μm at full field and of 71.86 μm on-axis. This puts a minimum of 80% of the optical energy of the image of an infinitely small point source within a circle of less than 200 μm diameter. This is a significant improvement over one existing cytometry lens design's 442.6 μm full field and 365.2 μm on-axis spot sizes and 800 μm diameter circle energy (at 80% energy).

Other design parameters for the lens optimization software include a magnification of at least 10×, and preferably between 10.5× and 11.5×, and a back focal length of 127±2 mm (as seen for surface **15** in the table, a back focal length of 126.731 mm is obtained for the present embodiment), and a wavelength range at least from 400 mm to 700 mm (the entire visible light range). The total length and lens barrel diameter should be as small as possible, i.e. less than 57 mm and 41 mm respectively, since space near the flow cell is in high demand in cytometry instruments. A lens length of 47.2 mm (combined thickness for surfaces **3** to **14**) and a maximum aperture radius (for less surfaces **13** to **15**) of 17 mm show that these size goals have been met.

The lens is seen to comprise (a) a nearly hemispheric plano-convex crown glass lens (surface **308** through **305** in the above table including the cemented plate of identical

material added for handling) with its planar side **303** closest to the cytometry flow cell and its convex surface **305** having a radius of curvature in a range from 3.5 to 5.5 mm (4.66 mm in the present preferred embodiment); (b) a pair of positive meniscus lenses (surfaces **306** to **309**) with their concave sides **306** and **308** closest to the flow cell (i.e. on the object side of the lens system) and with the surfaces **308** and **309** of the second meniscus lens being less sharply curved than the corresponding surfaces **306** and **307** of the first meniscus lens, which are in turn less sharply curved than the convex surface **305** of the plano-convex lens; and (c) a pair of positive doublet lens elements (surfaces **310**–**315**) to compensate for chromatic aberrations from the first three lens elements. The near hemispheric shape of the plano-convex lens (total axial thickness of the lens plus the attached plate of identical crown glass material being 4.715 mm compared to the 4.66 mm radius of curvature of the convex surface **305**, of difference of less than 1.2%) gives the lens system its large field of view. The convex radius of curvature range provides for a long working distance of at least 1.75 mm (about 2.2 mm in the present embodiment). Use of two meniscus lenses, and also the use of crown glass material (refractive index less then 1.55) for both the meniscus lenses and the plano-convex lens, reduce aberrations, which are generally proportional to the square of the amount of light bending at each refractive surface. The lower aberrations provide improved resolution, as indicated above the image spot size and circle energy. The doublets are not achromats themselves, but are over compensated so that the chromatic aberrations are reduced for the entire lens system.

What is claimed is:

1. A detector apparatus for analyzing light emitted from a fluorescent material, wherein the light is collected by a light collector and formed into an output beam for analysis, comprising

a means for collimating said output beam, the collimated output beam having a projected optical axis,

a plurality of dichroic mirrors disposed along the projected optical axis in a manner separating light at each mirror into a reflected beam and a transmitted beam, wherein, at each mirror, one of the reflected and transmitted beams is a transfer leg carrying the beam further to the next dichroic mirror and the other is a leg carrying light to a detector, wherein a majority of the dichroic mirrors receives light from a reflected beam coming from another dichroic mirror.

2. The apparatus of claim **1** wherein all of the dichroic mirrors except one receive light from a reflected beam coming from a dichroic mirror.

3. The apparatus of claim **1** wherein the number of dichroic mirrors is at least four.

4. The apparatus of claim **1**, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output beam at an angle of 20° or less.

5. The apparatus of claim **1**, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output beam at an angle between 5° and 20°.

6. The apparatus of claim **1**, wherein a plurality of dichroic mirrors are angled relative to an optical axis of the transfer leg or the output beam at an angle of about 11.25°.

7. The apparatus of claim **1** wherein said detectors are arranged in a polygonal pattern having a first circumference and said dichroic mirrors are arranged in a polygonal pattern having a second circumference smaller than said first circumference.

8. The apparatus of claim **7**, further comprising a plurality of filters arranged in a polygonal pattern having a third circumference, wherein a filter is associated with each detector and said third circumference is greater than said second circumference but less than said first circumference.

9. The apparatus of claim **1** wherein said dichroic mirrors are arranged such that said transfer legs carrying said beam from one dichroic mirror further to the next dichroic mirror follow a zigzag pattern.

10. The apparatus of claim **1** wherein said means for collimating said output beam receives light by means of an optical fiber.

11. The apparatus of claim **1**, wherein said detectors are photomultiplier tubes or semiconductors.

* * * * *

EXHIBIT 3

US007201875B2

(12) **United States Patent**
Norton et al.

(10) **Patent No.: US 7,201,875 B2**
(45) **Date of Patent: Apr. 10, 2007**

(54) **FIXED MOUNTED SORTING CUVETTE WITH USER REPLACEABLE NOZZLE**

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(73) Assignee: **Becton Dickinson and Company**, Franklin Lakes, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 835 days.

(21) Appl. No.: **10/259,332**

(22) Filed: **Sep. 27, 2002**

(65) **Prior Publication Data**

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(51) **Int. Cl.**
G01N 33/00 (2006.01)

(52) **U.S. Cl.** **422/73**; 422/82.05; 422/82.09; 422/102; 356/335; 356/336; 356/337; 356/338; 356/441; 356/442

(58) **Field of Classification Search** 422/68.1, 422/73, 82.05, 82.08, 82.09, 99, 100, 102; 356/335, 336, 337, 338, 441, 442
See application file for complete search history.

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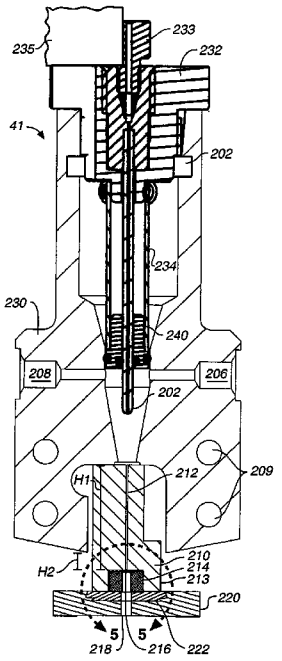
Assistant Examiner—Dwayne K Handy
(74) *Attorney, Agent, or Firm*—Douglas A. Petry

(57) **ABSTRACT**

A flow cell and flow cytometer in which a nozzle at the end of a flow channel is disposed on a removable substrate held at a registered location on a flow cell. Other elements including illumination optics, light collection optics, and the flow cell may then be positioned at fixed locations and would not require subsequent periodic adjustment. The registered location for positioning the nozzle allows removal and replacement of the nozzle key with the nozzle subsequently positioned in the identical location.

26 Claims, 9 Drawing Sheets



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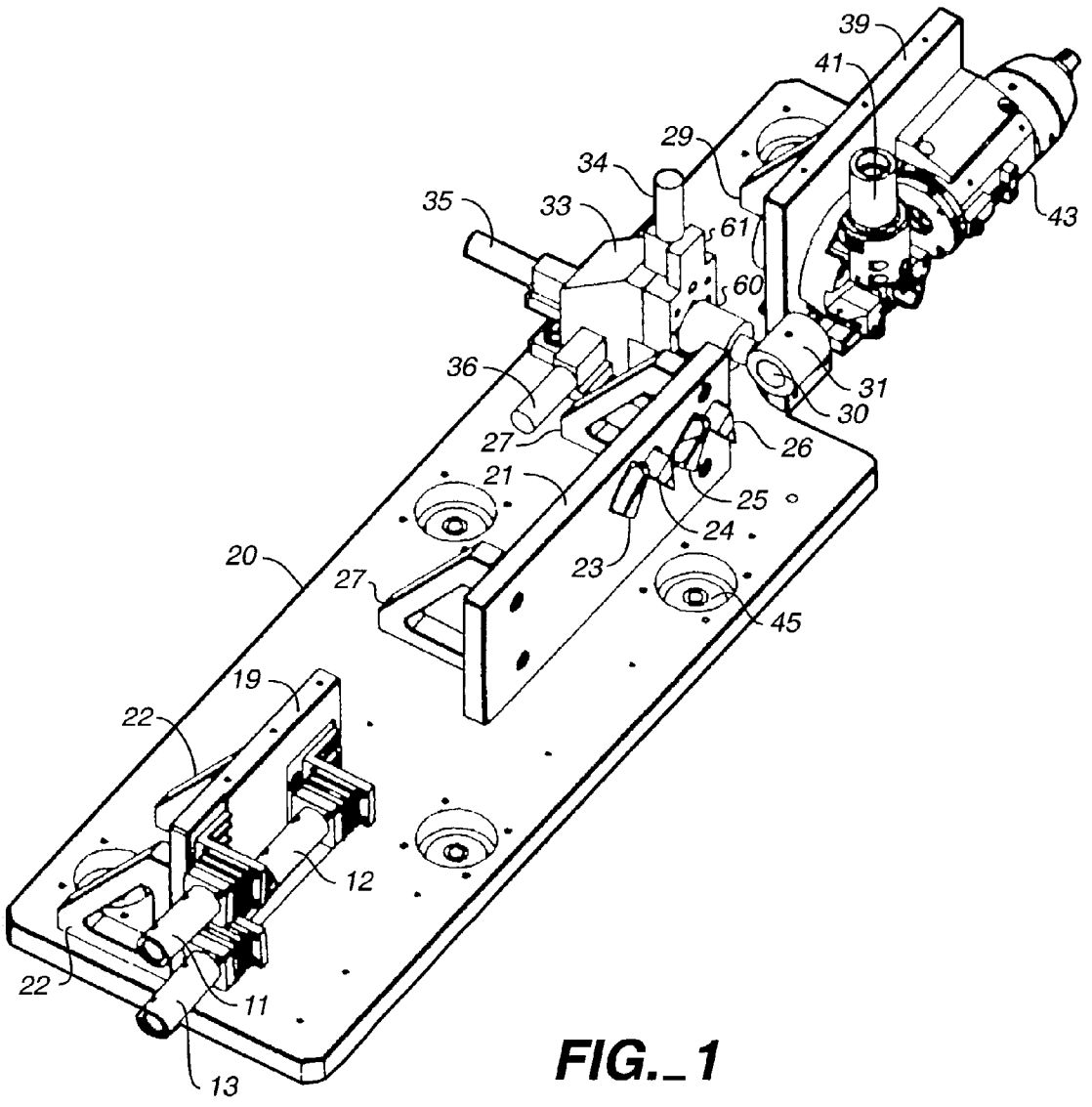
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FIG._1

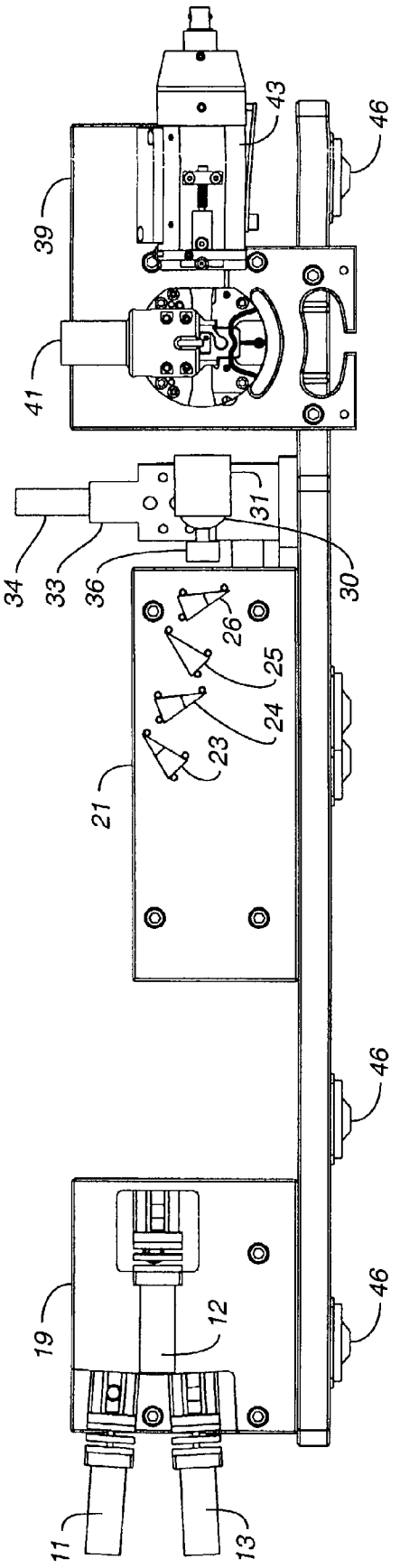

FIG._2

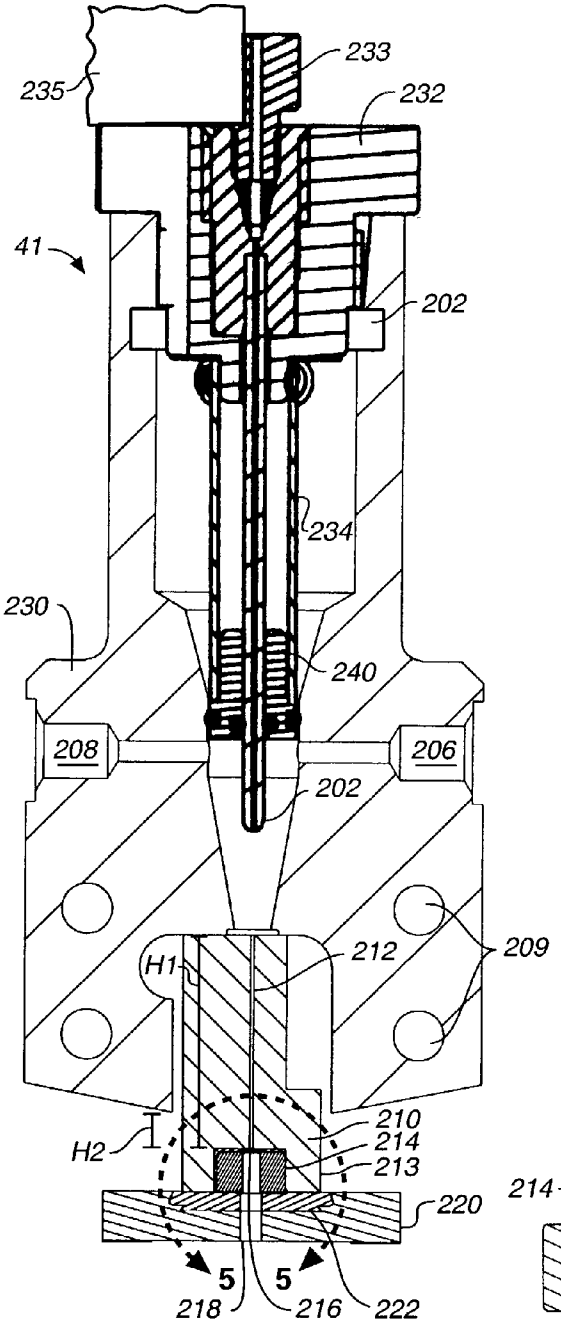
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FIG._3

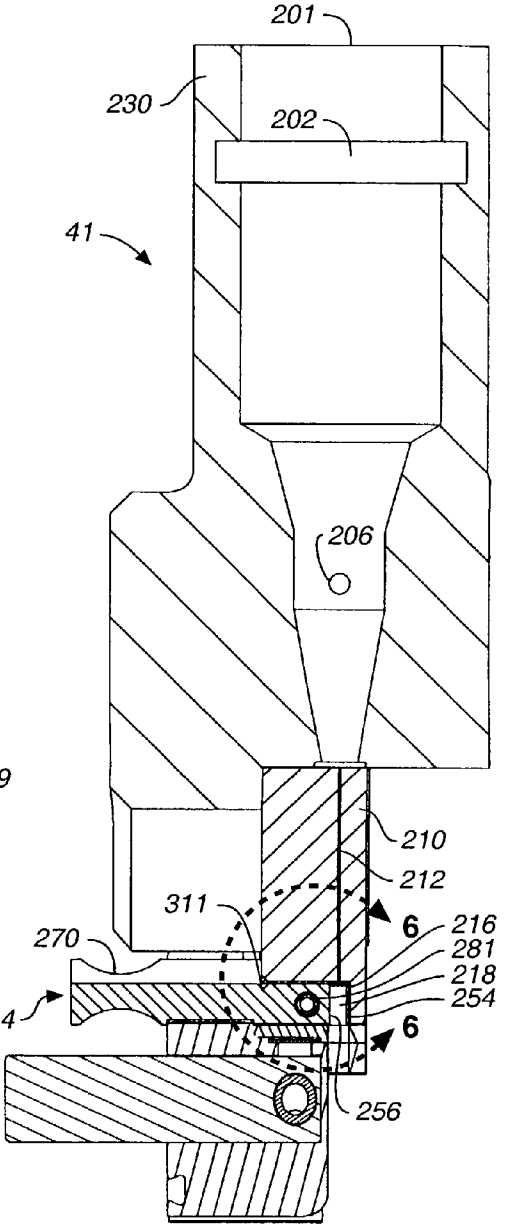
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FIG._4

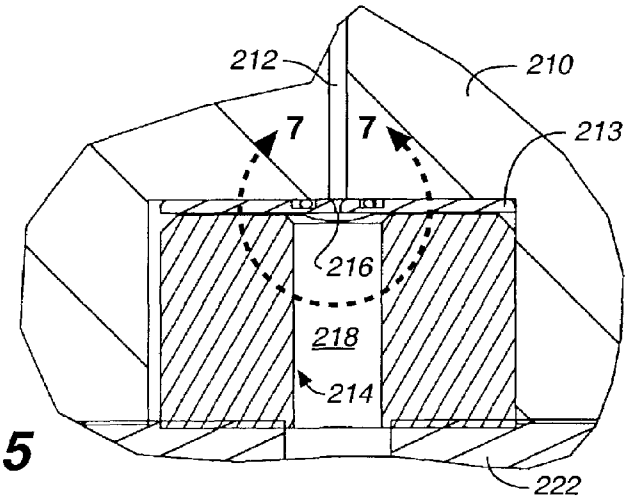

FIG._5

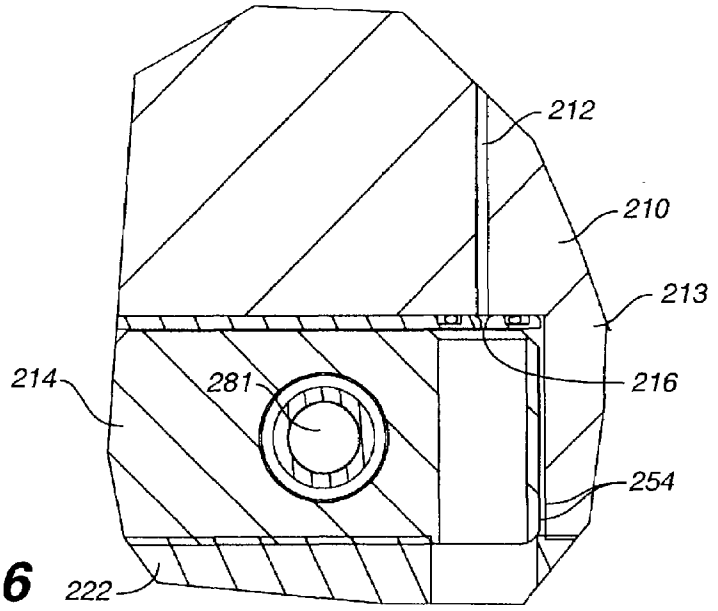

FIG._6

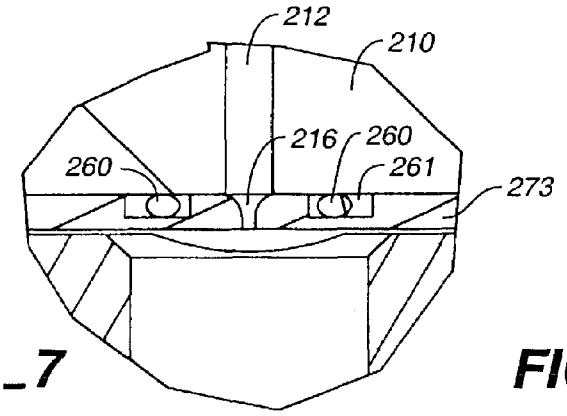

FIG._7

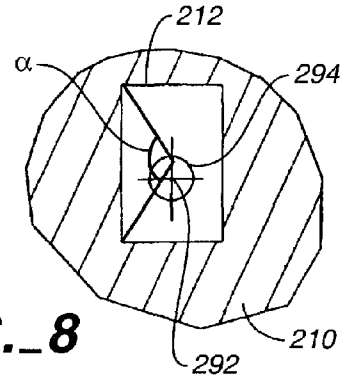

FIG._8

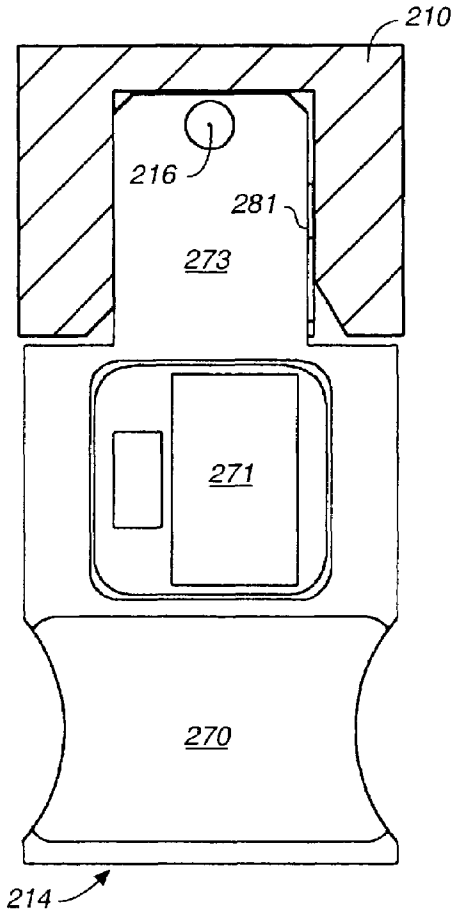
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FIG._9

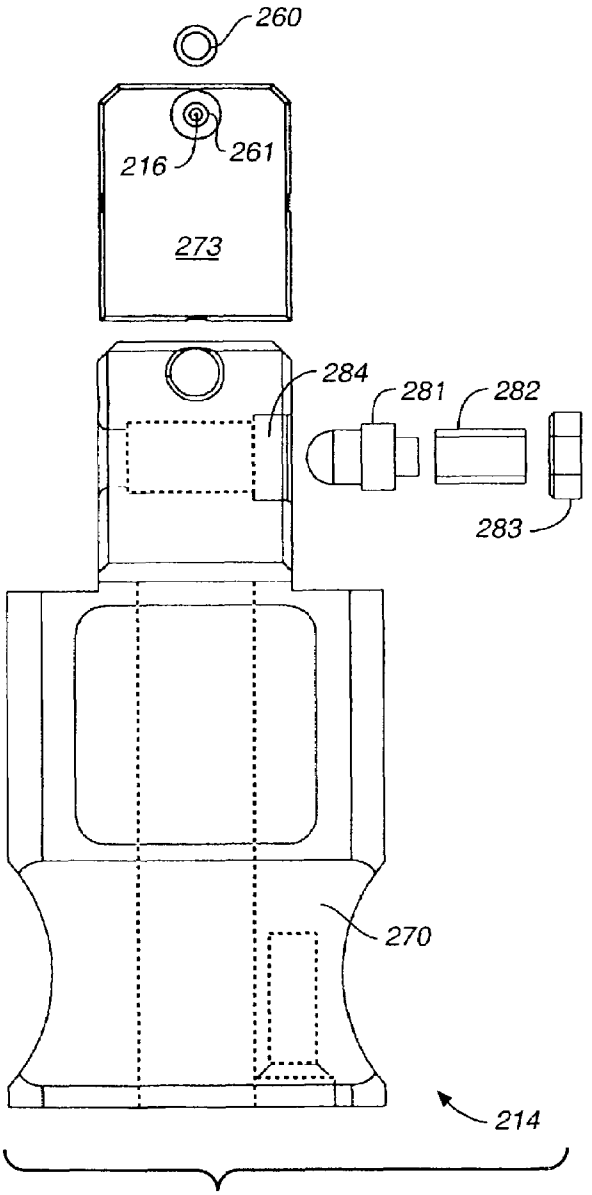
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FIG._10

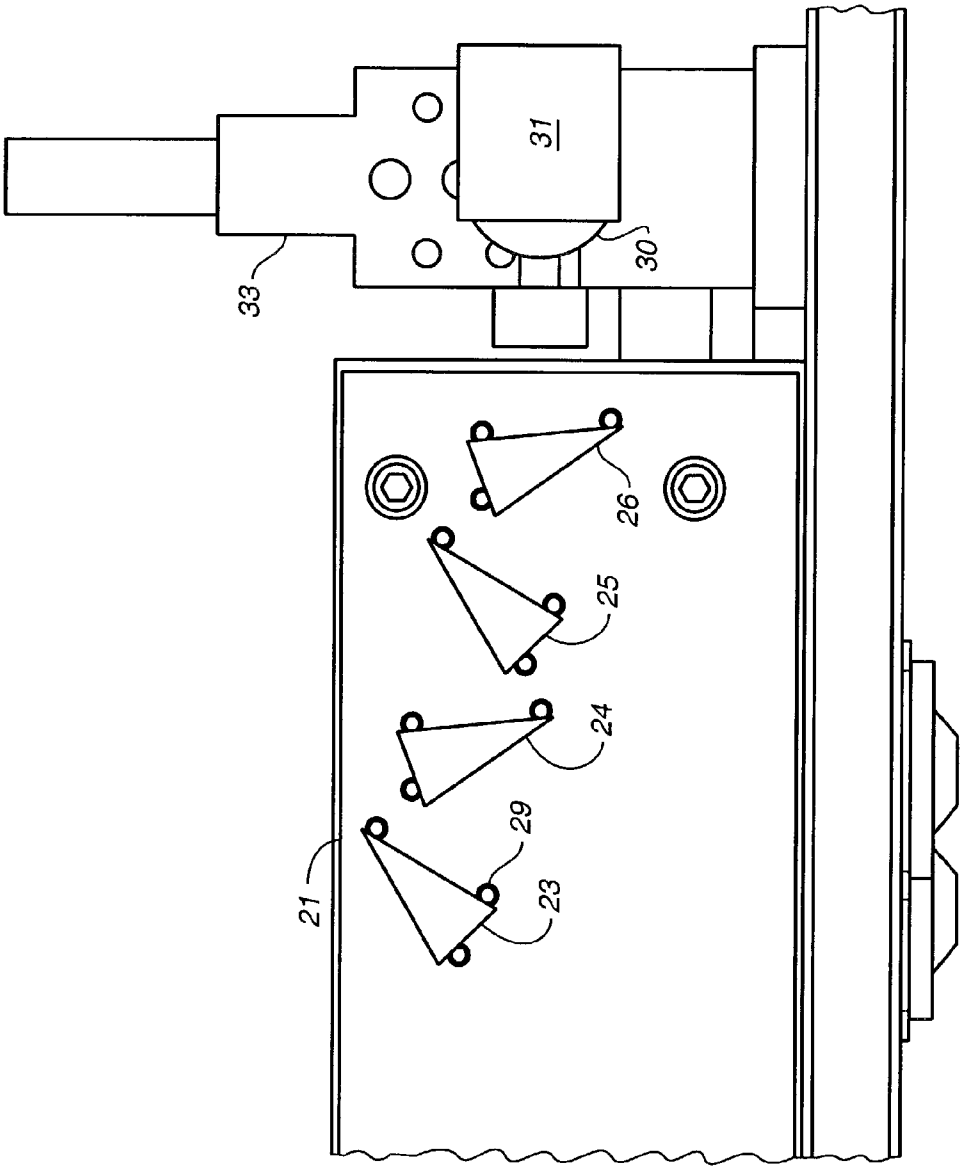

FIG._11

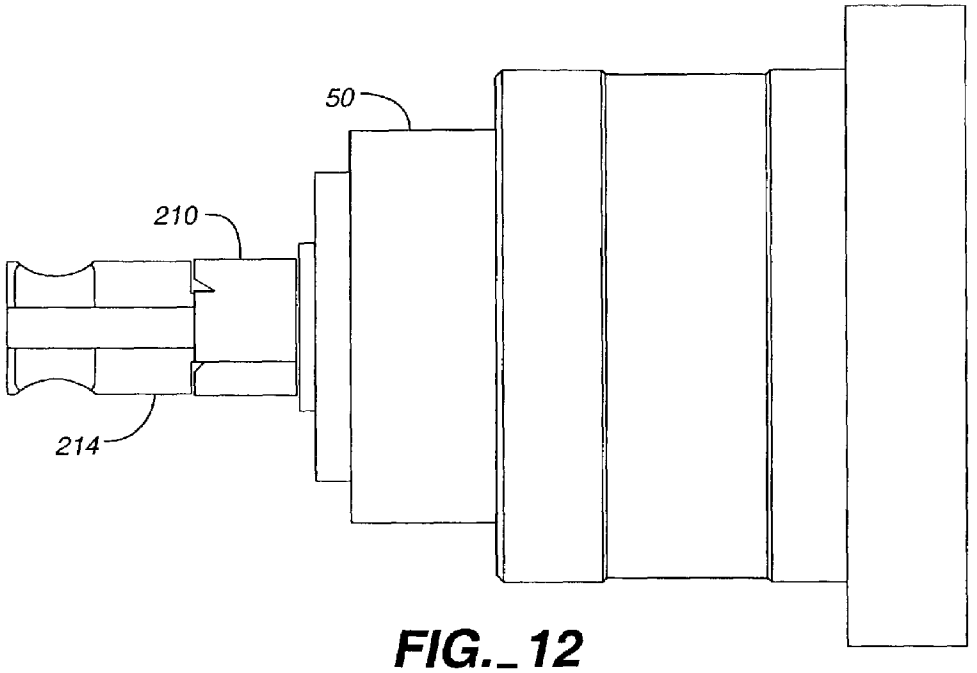

FIG._12

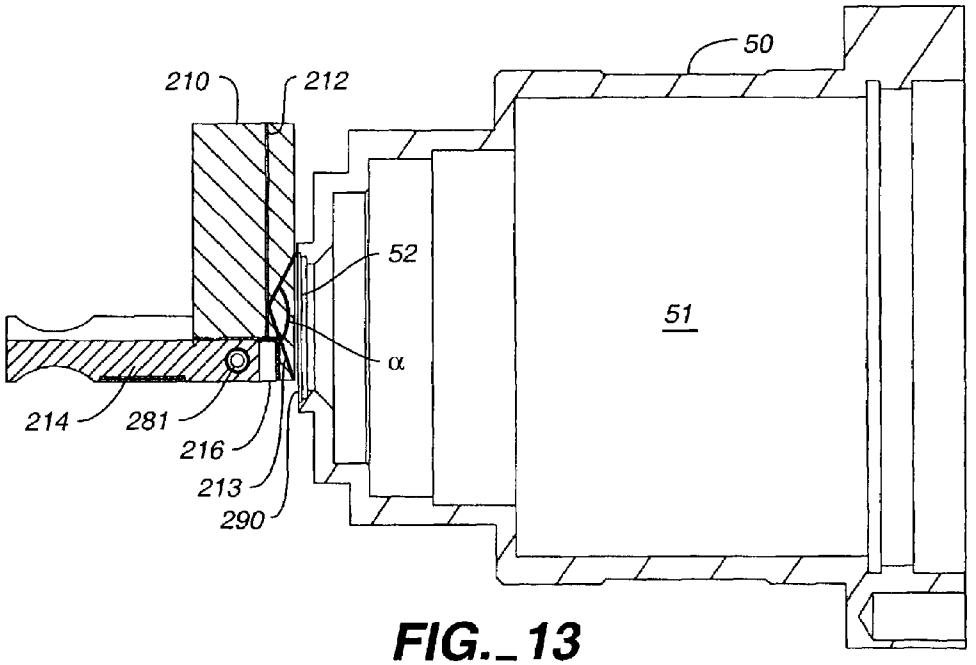

FIG._13

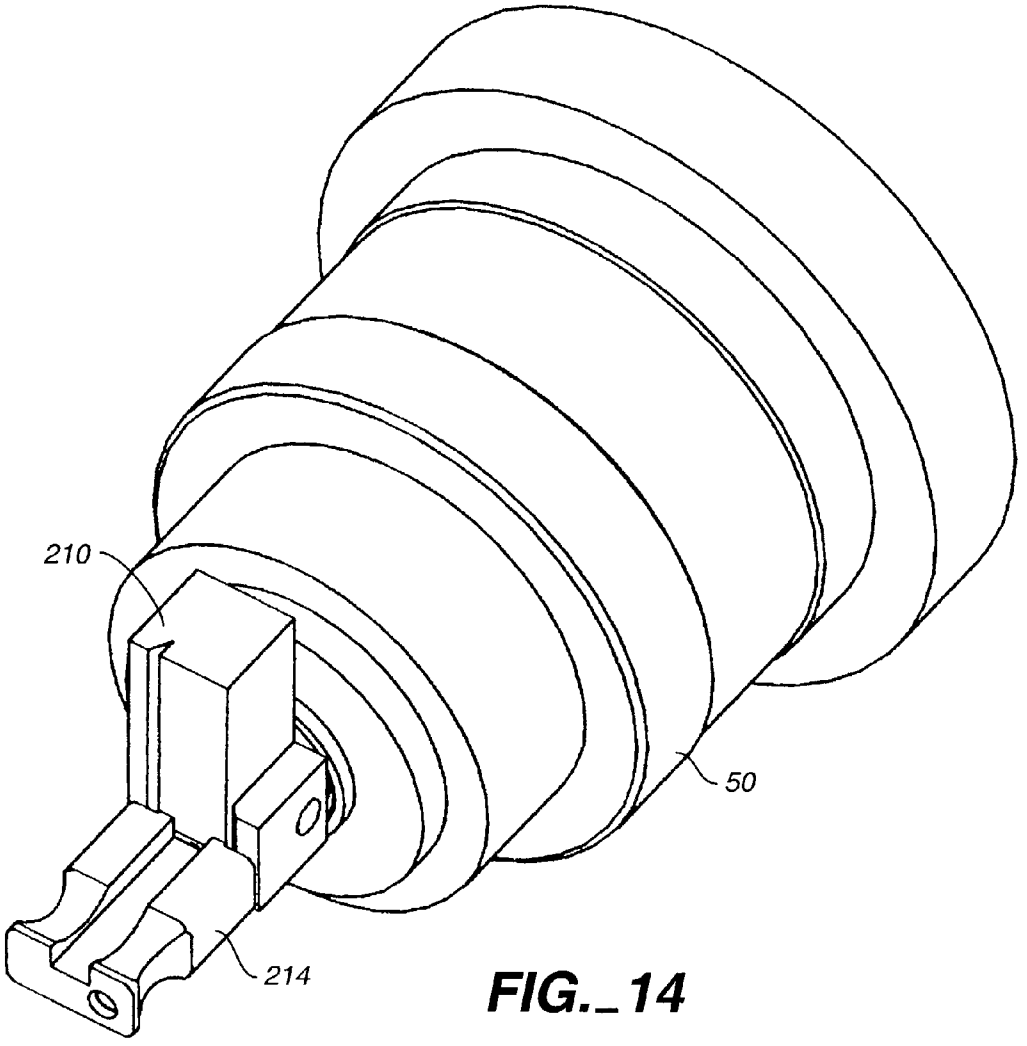

FIG._14

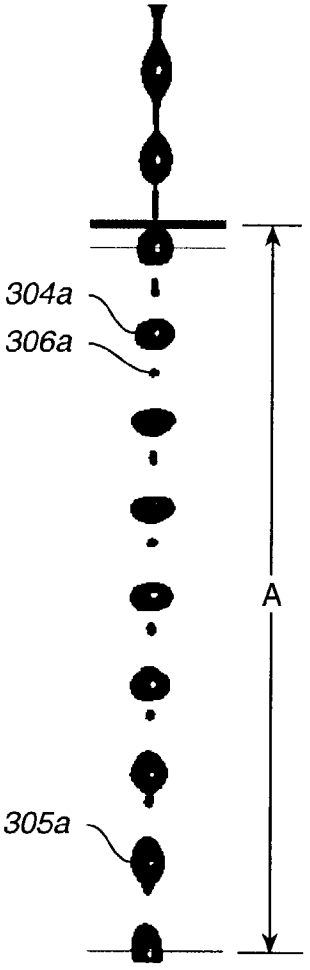

FIG._15A

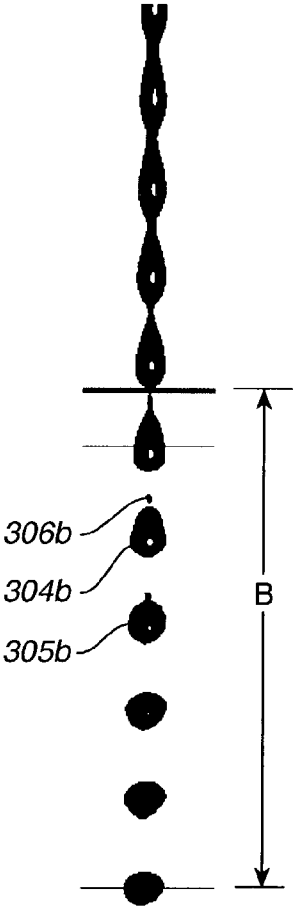

FIG._15B

FIXED MOUNTED SORTING CUVETTE WITH USER REPLACEABLE NOZZLE

TECHNICAL FIELD

The present invention relates to flow cytometry.

BACKGROUND OF THE INVENTION

Flow analysis has proven to be an important technology for the analysis of discrete targets. The applications of this technology include cellular assay to investigate a variety of cellular features including DNA content, specific nucleic acid sequences, chromatic structure, RNA content, specific antigens, surface receptors, cell morphology, DNA degredation and other assay targets. The targets of a flow cytometer may be multicellular organisms (e.g. microfilaria), cellular aggregates, viable cells, dead cells, cell fragments, organelles, large molecules (e.g. DNA), particles such as beads, viral particles or other discrete targets of this size range. The term "cells", as used throughout, is used to refer to such discrete targets. This technology has a number of different applications, including diagnostic, clinical and research applications.

Flow cytometry measures targets flowing through an analytical region in a flow cell. In the flow cell a core stream is injected into the center of a sheath flow stream flowing at a constant flow rate. The core stream is a liquid sample, which may be injected from a sample tube. Injection generally requires insertion of an aspiration tube into the sample tube and pressurization of the head above the liquid in the sample tube such that sample liquid is pressure driven from the sample tube into the injection tube.

The flow stream is directed into a tapered portion of the flow cell body and through an analytical region. In one design, the stream is directed through a nozzle and analyzed in air. In a second design, the stream is directed through a channel for analysis.

Analysis takes place by optical interrogation of particles as each particle passes a detection region. In most systems, one or more laser beams are directed by steering mirrors and illumination lenses through the analytical region. If more than one laser are used, a dichroic stack may be used to combine the beams and direct the beams through the stream to be analyzed.

Some of the light passing through the analytical region will be scattered by particles. Detectors measure the intensity of forward and side scatter. In addition, the illumination beam will excite fluorescence from target particles in the flow stream that have been labeled with a fluorescent dye. Emitted fluorescence is collected by a collection lens and transmitted to detection optics. The detection optics separate the collected light (e.g. using filters and dichroic mirrors) into light at specific wavelengths. Light at specific wavelengths, or within specific wavelength ranges, are detected by individual light detection devices (e.g. photomultiplier tubes). The signal from the various detectors is sent to a data processor and memory to record and characterize detection events.

In addition to analysis of particles, flow cytometer systems may also be designed to sort particles. After leaving the optical analysis region, the flow stream may be separated into droplets. One common method of droplet generation is to vibrate the nozzle from which the flow stream emerges. This may be done by vibration of the nozzle alone, or vibration of the entire flow cell. The resultant separated droplets adopt a spacing which is a function of the stream velocity and the vibration wavelength. Droplets containing the target of interest are charged by a charging device such as a charging collar. The charged droplets are directed between two charged deflection plates, which angularly deflect charged droplets. The deflected droplets are then collected in containers positioned in the path of falling deflected particles.

Known flow cytometry similar to the type described above are described, for example in U.S. Pat. Nos. 3,960,449; 4,347,935; 4,667,830; 5,464,581; 5,483,469; 5,602,039; 5,643,796 and 5,700,692. All references noted are hereby expressly incorporated by reference. Commercial flow cytometer products include FACSort™, FACSVantage™, FACSCount™, FACScan™, and FACSCalibur™ systems all manufactured by BD Biosciences, the assignee of the instant invention.

The described system presents a number of advantages for the analysis of particles (e.g. cells), allowing rapid analysis and sorting. However a number of limitations to the system exist.

Alignment

The system requires precise alignment of various elements to function properly. The lasers must be precisely positioned to properly direct light to the objective. To aid in this positioning, the laser or other illumination source is commonly mounted on an x-y-z stage, allowing three-dimensional positioning of the laser. The steering mirrors for the laser beams must be precisely positioned to properly direct the illumination beam to the objective. This generally requires that the mirrors be mounted to allow for angular adjustment. The illumination lens system must be exactly positioned such that the illumination lens focuses the illumination light onto the target area. This lens is also generally mounted such that it can be repositioned along the x-y-z axes.

The flow cell must be positioned such that the angle at which the illumination beam impinges the flow stream and the distance from the flow stream to the illumination lens does not change. Commonly the flow cell is mounted on a stage, which allows x-y-z positioning of the flow cell. In addition the stage holding the flow cell may also allow for angular repositioning of the flow cell (e.g. α and θ positioning). This angular adjustment is critical for sorting, which requires precise prediction of the sort stream direction. In addition, the optics used for detection of scattered light and fluorescence also must be properly aligned.

The stream in air jet must also be aligned, to ensure that the stream in air is directed in the intended direction. This alignment is effected by angular rotation of the flow cell. This alignment is additionally important if the optical interrogation of the stream takes place in a stream-in-air. The alignment procedure for a stream in air system requires first locating the stream-in-air with respect to both the illumination and the light collection optics and then focusing each of these components on a location within the stream in air.

Alignment requires user time and considerable user expertise. At times it is difficult to determine which element requires adjustment. Set up of the instrument generally requires a diagnostic of alignment with elements realigned by repositioning as needed. This occurs at least once a day, more frequently if an element is replaced or removed. Realignment necessitates both instrument down time and user time and expertise. The time required to perform the alignment procedure is highly dependent on both the con-

dition of the system and the skill of the operator. In addition, the need for constant realignment reduces the repeatability of system performance.

A few attempts have been made to address the problem of the need for repeated alignment of some elements of a flow system. U.S. Pat. Nos. 5,973,842 and 6,042,249 to Spangenberg disclose an optical illumination assembly for use with an analytical instrument. This assembly may include an illumination source (e.g. a laser), a spatial filter, a beam shaping aperture and a focus lens. All elements are illumination optical elements, not the flow cell or light collection elements. Each component is mounted on a plate, frame or mounting cylinder, which in turn are mounted on a platform. Each of the plates or frames is movable along two axes by micrometer adjustments using adjusters with opposing spring plungers. Following an initial adjustment, the plates or frames are secured into a fixed location using screws or other devices to fix the plates or frames into place. The adjusters or springs are removed once the frames or plates are secured. The focus lens would be mounted such that it would be moved along 3 axes (x-y-z movement) and subsequently also be fixed into a location. This allows fixation of the light generation and illumination optics. However, the cuvette would still be adjusted to be positioned at the focal spot of the illumination. This would be required on a routine basis.

U.S. Pat. No. 4,660,971 discloses an illumination configuration in which a focus lens is in contact with a flow cell. A spring biases the lens against a housing, positioning the lens at a selected focal length from the flow cell. This maintains a relative axial position between the lens and the flow cell.

These references, while providing a method in which some of the issues relating to the alignment of the illumination optics are addressed, do not provide a method in which the flow cell and the light collection optics may also be fixed. Fixing all of these elements significantly further simplifies the alignment of the instrument.

Illumination Power

A number of different features in a common flow cytometer setup result in loss of illumination intensity or loss of intensity of collected light. To compensate for these losses generally requires increased illumination power. This requirement for increased power requires expensive and bulky liquid-cooled lasers that provide sufficient power to overcome losses and still allow sensitive target detection. These sources of loss include:

1. Optical interrogation using a stream-in-air. The gross cylindrical geometry of a stream of liquid in air acts as a lens both reflecting and refracting illumination light. This high index of refraction is more pronounced in smaller diameter streams. This refraction makes illumination less efficient and distorts light scatter. To mitigate this effect of scatter distortion an obscuration bar is positioned between the stream in air and the light scatter detector. In some systems, this rectangular obscuration bar may be rotated to block additional amounts of light scatter across a greater area, blocking additional light from narrow angles from reaching the scattered light detectors.
2. Use of dichroic mirrors to combine illumination beams. Each dichroic mirror is not able to perfectly reflect or transmit a light beam. As the beam is reflected or transmitted some light is lost. This loss ranges from 10–20% of beam power (5–10% if beam is reflected; 10–15% loss for transmission through a dichroic mirror), more if the dichroic is not perfectly aligned. A laser beam that is reflected by a steering mirror through two dichroic mirrors to combine three beams could lose 40% or more of the laser's power.
3. Losses in collection of fluorescence. The amount of collected fluorescence can be limited by the optical properties of the flow cell and the collection lens. The geometry of the flow channel and the flow cell define the numerical aperture from which the system is able to collect light. The transition from the flow cell to the collection lens could allow refraction of light and loss of signal as the emitted fluorescence travels through the flow cell, into air, and then into the collection lens. The high index of refraction during material transition results in the loss of collected light. In some systems this loss is mitigated by physical coupling of the flow cell to the collection lens. However, this coupling would be greatly simplified if the flow cell were in a fixed location.

Droplet Generation

Droplet generation has required vibration of some part of the flow cell, generally either the nozzle or the entire flow cell. Vibration of the entire flow cell can result in alignment difficulty as well as additional light scatter created by the vibration. In addition, if the optical analysis is performed in a stream-in-air, the drop-drive perturbations cause undulations on the free surface of the stream. This causes a constant alteration of the light paths into and out of the jet of liquid, making measurement of scatter and focusing of the illumination beam more difficult.

U.S. Pat. No. 6,133,044 provides one alternative to the vibration method of droplet generation. This reference describes a device in which an oscillator is included within the nozzle volume or otherwise is undirectionally coupled to the sheath fluid. The tapering of the nozzle amplifies the oscillations, which are transmitted as pressure waves through the nozzle volume to the nozzle exit. This results in the formation of droplets. The nozzle is directionally isolated to avoid vibration of the entire flow cell or nozzle and limit the oscillations to forming pressure waves in the flow stream.

Optics Positioning Limitations

Ideally, the flow cell would be materially joined to the light collection optics to prevent the loss of collected light. One of the greatest losses of collected light occurs due to the transition between different materials that each have a different idex of refraction of light. The light refraction between different materials (e.g. air and glass) may be significant and the resultant light refraction makes the collection and measurement of scattered or fluorescent light difficult. This is mitigated by joining the flow cell to the light collection lens. However for the flow cell and the light collection lens to be coupled by a physical material would require that the two elements remain in a fixed location.

In addition, the need to guard the flow cell from damage (e.g. scratching of surfaces through which light passes) presents another motivation for keeping the flow cell at a fixed location.

Flow Cell Positioning Limitations

Sorting flow cytometers generate a stream of droplets in air and subsequently sort droplets containing target particles. The droplet stream is generated from a flow nozzle positioned at one end of a flow channel. A large degree of uncertainty in the nature of the stream of droplets is a common result of the way in which the nozzle is located to the flow channel. Most flow designs rely on the "self-aligning" tendency of a female conical structure at the

nozzle inlet, which mates with an edge on a cylindrical structure at the flowcell outlet (i.e. the outlet of the flow channel). Typically an o-ring makes a seal between the nozzle conical structure and the flow channel cylindrical structure.

However, there are a few problems inherent with this approach. First, the o-ring has a compliance that aggravates the axial and angular tolerance stack-up associated with locating a conical surface about a circular arc. Second, the angular location of the nozzle about the axis of the flow cell is arbitrary. Third, the angular location of the o-ring about the axis of the flow cell is arbitrary. The first noted problem makes it difficult for a user to duplicate the mounting of the nozzle to a previous mounting configuration. The second and third noted problems make it impossible. Because the angular location of the nozzle and the o-ring are arbitrary, the nozzle is not formally constrained with respect to the flow channel (or the cuvette) through which the flow cell extends.

U.S. Pat. Nos. 6,263,745 and 6,357,307 to Buchanan et al. disclose a nozzle for sorting flat samples. This nozzle seats in a cylindrical recess in the flow cell. U.S. Pat. No. 6,133,044 discloses a removable nozzle for use with a flow cytometer. The nozzle seats in a cylindrical recess in the flow body and is held against a lip. An annular nut secures the nozzle to the body of the tapering flow cell. An o-ring positioned between the nut, the nozzle and the tapering flow cell provides a means for ensuring the axial orientation of the nozzle.

Cell Sorting

Cell sorting requires precise coordination of event detection, droplet generation and droplet tagging. If these procedures become even slightly out of coordination, the incorrect droplets could be charged for sorting or the system could fail to collect the desired particles or cells. For stream-in-air analysis and sorting, this process is simplified because the droplet stream is optically analyzed, droplets are generated and droplets charged all in a stream in air. However, as noted earlier, the stream-in-air sorting produces a decreased signal from cells or particles sorted and the circular stream of liquid can cause both illumination light and scattered light to be reflected or refracted.

Sorting using a system in which analysis is done in a channel also presents challenges. When the liquid moves from an analysis channel and subsequently through the nozzle the velocity of the particles changes, as the liquid flow accelerates at the narrow nozzle. The coordination of flow must account for this change in flow rate.

U.S. Pat. No. 6,372,506 to Norton discloses an apparatus and method for determining drop delay. Drop delay is the time that elapses between detection of a target at an analytical region to the time at which a sorting condition (e.g. a charging potential) is applied to the droplet. As the droplets are formed they are analyzed to determine whether the drop delay is correct. The droplets are analyzed to determine if the target detected at an analytical region is within the droplet to which the sorting condition is applied.

As fluid enters a channel, flow over a short distance can be modeled as "slug flow", all liquid moves as a single front. This would be the case at the entrance of the neckdown region of the flow cell. As liquid moves along the length of the channel, the viscosity of the liquid produces a parabolic velocity profile. The velocity of the liquid flowing through the cuvette channel tube is fastest along the longitudinal axis of the tube. At the walls of the tube the fluid has no velocity. At any intermediate point between the walls and the center of the channel, the velocity of the fluid varies parabolically. This laminar flow results in a spreading of the distance between particles at different distances from the tube center as the particles move through the stream. Particles in the exact center of the stream will move faster than the particles closer to the edges of the stream.

The laminar flow produces a spread of particles as the particles move through the channel. This can make sorting particles optically analyzed in a cuvette channel more difficult. If the velocity of a particle changes as the particle moves through the channel and optical interrogation occurs in the channel, the velocity of the particle at the point of optical interrogation and the velocity of the particle at the point of exiting the channel through a nozzle will be significantly different. Since prediction of the position of the particle depends on knowing the velocity of the particle, sorting particles becomes much more difficult if the velocity of the particle changes.

It is an object of the invention to provide a flow cytometer that requires alignment less frequently, and most preferably only at an initial instrument setup.

It is a further object that this system allows for efficient illumination and collection of light.

It is a further object that the losses of illumination light be reduced to allow for lower power lasers to be used for illumination.

It is a further object to provide a system that is easier to use and provides robust system performance.

SUMMARY OF THE INVENTION

The present objects of the invention are achieved through a number of embodiments of the invention in which elements of a flow cell or flow cytometer system are designed for efficient light collection, efficient droplet production, and minimization of the need for user manipulation of the system.

In one embodiment the invention includes a removable nozzle key, which fits into a registered location on a flow cell at the end of a flow channel. Clogs are an issue: The customer-removable nozzle addresses this with no subsequent alignment required. The nozzle key may be inserted into a registered location on the flow cell such that the nozzle is precisely positioned. The nozzle key may be removed, cleaned, refit into its precise location.

Removal of the nozzle allows the flow cell to be attached at a fixed location on a system platform. If the flow cell position is fixed, other optics that must be positioned relative to the flow cell may also be fixed. This allows the illumination optics, the fluorescent light collection optics and the scattered light detection optics to also be in a fixed location.

The fixed illumination optics may include fixed optics for transfer of the illumination beams into the system and fixed optics for beam shaping and orientation. The optics for bringing the illumination light into the system could use optical fibers coupled into the system at fixed location mounts. The optics for shaping and orienting the beams could be refractive optics, which are less alignment sensitive than the mirrors used in prior systems for beam redirection and shaping.

The light collection optics may also be fixed. If the flow cell is fixed and the light collection optics is fixed, the flow cell may be materially coupled to the light collection lens, as by gel coupling. This lowers losses to refraction.

The design of the present system's elements aids in efficient light collection. In one embodiment, the cuvette containing the flow channel has sidewalls extending on three

sides of the cuvette below the plane containing the opening of the flow channel. Light emission from the flow channel may pass into the sidewalls and subsequently into the light collection optics. This allows for light collection from a greater numerical aperture than is seen in prior systems. This design also allows the optical analysis to take place quite near the bottom of the flow channel. This makes determination of the drop delay (needed for charging generated droplets for subsequent sorting) simpler. In addition, there is less variability between particles of different velocities. Many of these described features are independent embodiments of the present invention.

In another embodiment of the invention, a flow cell for a sorting flow cytometer is provided in which a removable nozzle is inserted into a registered position in which it is held at a fixed location in relation to the rest of the flow cell. This fixed position prevents the nozzle from either three-dimensional or rotational movement.

Flow cells include a sample delivery tube, at least one sheath flow port, and a channel for optical analysis. This channel may be part of a flow cell body, but preferably is a cuvette joined to the flow cell body. When the cuvette is joined to the flow cell body, the sheath flow and sample stream flows into the cuvette.

Flow cells for a sorting flow cytometer also include a droplet generator. The droplet generator would ether vibrate an element on the flow cell, such as the nozzle, cuvette or flow cell body, or would introduce a oscillating pressure wave within the flow cell body.

The removable nozzle is held on a substrate, such as a card or insertable key, which is fit into a registered position in which the substrate is registered against hard surfaces, allowing the substrate to be removed and replaced into a precise position.

In another embodiment, the sorting flow cell includes an oscillating droplet generator that transmits a pressure wave to the sheath flow fluid flowing through the flow cell. In this embodiment, droplets may be generated without a device for vibrating the flow cell, the cuvette, or the nozzle. A number of noted features, including the registered nozzle, may be included with this embodiment.

In another embodiment, the sorting flow cytometer flow cell includes a flow channel of rectangular cross-sectional dimensions. The shorter side of the channel would face the optical path of the illumination light directed by the illumination optics. The longer side of the channel would face the light collection optics. This configuration has a high numerical aperture for collection of emitted light. The channel may extend through a cuvette. The cuvette may have sidewalls that extend below the area of the nozzle, allowing a wider angle to collect emitted light. This configuration also allows an enhanced numerical aperture of collected light compared to systems lacking the sidewalls. Again, a number of noted features, including the registered nozzle, may be included with this embodiment.

In another embodiment, a flow cell component includes a flow cell for a sorting flow cytometer and light collection lens. The flow cell is joined to the light collection lens by a light transmissive material. In this manner there is no transition into air as light moves from the flow cell to the light collection lens. This reduces the loss due to the change in index of refraction as light moves from the flow cell, into air, and then into the collection lens. In prior non-sorting flow systems, cuvettes could be joined to the collection lens. However, in sorting systems, the need to clear the nozzle and vibrate the flow cell made joining the collection lens and the flow cell inadvisable. In the present invention, these limitations have been overcome, and the advantages of joining the flow cell to the collection lens are achieved.

In another embodiment, the flow cell for a sorting flow cytometer includes a nozzle held at a hard registered location that is off center from the longitudinal center of the channel, this is possible because the nozzle may be removed and reinserted into a precise location. This provides favorable conditions for the formation of droplets and the merger of satellite droplets into parent droplets.

Each of these embodiments represent a component which may be independently produced. Alternatively, each component (flow cell or flow cell with optics component) may be part of a flow cytometer system. Such a system would include light collection optics and illumination light direction optics. The system could also include input optics that would allow illumination light sources to be coupled to the system. Alternatively, the system could be produced with the illumination optics as an already fixed part of the system. In addition, the features of each embodiment may be incorporated with the features of other embodiments if desired.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective view of a flow cytometer system incorporating features of the present invention.

FIG. **2** is a side view of the system of claim **1**.

FIG. **3** is a frontal cross section of a flow cell, nozzle and nozzle support platform.

FIG. **4** is a side cross section of the device of FIG. **3**.

FIG. **5** is a detail of the nozzle and flow cell shown in FIG. **3**.

FIG. **6** is a detail of the nozzle shown in FIG. **4**.

FIG. **7** is a detail of the nozzle from FIG. **5**.

FIG. **8** is a cross section detail illustrating positioning of the nozzle in the flow channel.

FIG. **9** is a top view of the nozzle card.

FIG. **10** is an exploded view of the nozzle card.

FIG. **11** is a frontal detail view of the light collection optics of FIG. **2**.

FIG. **12** is a top view of the nozzle card, flow cell, and fluorescence collection lens.

FIG. **13** is a side cross sectional view of the devices shown in FIG. **12**.

FIG. **14** is a perspective view of the devices shown in FIG. **12**.

FIG. **15**A is a view of droplet formation.

FIG. **15**B is a view of droplet formation using the technology of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention, significant advantage is derived from a configuration in which a number of the optical elements may be fixed with respect to the flow cell. This advantage arises from the extent of directional stability afforded by the nozzle, which the user may remove and replace and which is self-aligning. The nozzle is insertable in the flow cuvette at a location where the nozzle is registered in place. This registration allows the nozzle to be inserted and positioned such that the nozzle is constrained both as to translation and rotation. Because only the nozzle is movable, the flow cell may be fixed, and does not need to be positioned on a stage that may be angularly or directionally repositioned. As a result, no removal or replacement of the flow cell is required and the user will not have to adjust

or realign the flow cell assembly to align the stream of droplets with a required direction for sorting.

Because the flow cell and flow channel never need to be moved, the other optical elements that must be focused or positioned relative to the flow cell now may be fixed as well. This fixation may be location of the flow cell on one fixed plate and the location of other optical elements on one or more additional fixed plates, with each plate in a defined positional relation to any other plate. Alternatively, the flow cell could be materially linked to other optical elements, such as by physical joining of the flow cell with the collection optics. Materially joining the flow cell to the light collection optics allows reduction of the index of refraction between material transitions and allows more efficient collection of light.

The present configuration minimizes tolerance stack-up. Only the fabrication tolerances of two mating elements, the nozzle and the receiving flow cell body can contribute to the stack-up. These are the only two elements that would be moved in relation to the other. An intermediate locating element between the nozzle and the cuvette would, at least, double the tolerance stack-up and adversely affect stream stability.

With reference to FIGS. **1**, **2** and **11** the system allowing fixed position mounting of the elements is shown in a perspective view. The lasers (not shown) produce illumination light, which is directed through an optical fiber, linked to the system by mounts. The first, second and third optical fiber mounts **11**, **12**, and **13** respectively each receive an optical fiber bringing illumination light from one laser. Optical fiber mounts are mounted on plate **19** that is secured to platform **20** by braces **22**. Braces **22** ensure that plate **19** will be maintained in a fixed position. Light from optical fibers coupled to mounts **11**, **12**, and **13** is directed through illumination refracting optics.

A series of prisms are used to combine the illumination beams into illumination light having specific properties. At the point of illumination, it is preferred that the illumination beams be elliptical, concentrating the illumination energy at the central location of the core of the flow stream. As the light is directed through prisms **23**, **24**, **25** and **26** the illumination beams are differentially refracted by the prism such that the illumination beams are redirected and aligned at the illumination location within a flow cell. Prisms **23**, **24**, **25** and **26** are mounted on plate **21**, which is secured to platform **20** by braces **27**. The mounts for both the laser couplings and the prisms may be adjusted once at the setup of the system, positioning each element in a fixed location. This may be performed by either adjusting the mounts for each element, or repositioning the plate on which elements are mounted so that a number of elements are moved together. The prisms are less sensitive to angular misalignment and are more thermally stable. Both of these features aid in allowing set position of this element.

The illumination beams pass through the illumination beam combining optics and through illumination lens **30** held in illumination lens mount **31**. Illumination lens mount **31** is positioned on arm **60** secured to face **61** on lens positioning stage **33**. Micrometer **35** extends to arm **60** to allow movement of lens mount **31** along the z-axis. Plate **61** is movable by micrometer **34** to allow movement of the illumination lens mount along the y-axis. Micrometer **36** is mounted through lens positioning block **33** to allow movement of lens mount **31** along the x-axis. In combination, positioning micrometers **34**, **35**, **36** allow lens mount **31** to be repositioned in the x, y, and z directions. Lens mount **31** is mounted on block **33**, which is mounted on platform **20**. Plates **19** and **21** and block **33** are each at a separate location on platform **20**. Each of these elements may be separately adjusted initially at installation for alignment.

The focused illumination beams pass through the illumination lens held in lens mount **31** and through the optical analysis region of the flow channel in flow cell **41**. As particles pass through the flow channel and cross the illumination light beams, light will be scattered and fluorescence will be excited. Scattered light will be detected by forward light scatter detector **43**. Emitted fluorescence as well as large angle scattered light will be collected by emission collection optics. Flow cell **41** and forward light scatter detector **43** are mounted on plate **39**. Plate **39** is held on platform **20** by brace **29**. Platform **20** is supported on feet **46**. Isolation mounts **45** allow mounting of platform **20**.

In the system of FIG. **1**, the optics for bringing in light, redirecting the beams into the desired illumination orientation, collection of the scattered and emitted fluorescence are all held in a fixed position. In addition, the flow cell is also held in a fixed position. These elements are each mounted on a plate and need to be aligned initially when the instrument is installed. Routine realignment of these elements will not be required. The illumination lens could be routinely realigned. However, alignment of a single element greatly simplifies the time and difficulty of alignment.

It should be realized that a number of the elements of this system have independent utility. For example, simply having a fixed location flow cell and light collection optics allows joining of the flow cell to the collection lens to more efficiently collect illumination light. Fixing the mount location of the beam redirection optics also saves user time and provides a method of combining illumination beams into a single illumination beam without using mirrors (and the attendant loss of light characteristic of mirrors).

The term "fixed location" as used herein refers to an element which does not have a means for user adjustment. This element at a fixed location would be aligned initially (generally at instrument set up) and not require further alignment.

"Illumination input optics" refers to optical elements that allow introduction of light to a system (e.g. optical fiber mounts).

"Illumination beam directing optics" are optical elements that redirect and reshape the illumination beams (e.g. serial prisms). Illumination beam directing optics may or may not include a focus lens.

"Light collection optics" refers to optical elements disposed to collect emitted or scattered light from the flow channel. In flow systems such optics generally include a fluorescence and wide angle scatter collection lens and a forward scatter detector.

The ability to have a fixed position illumination input optics, illumination beam directing optics, flow cell, and light collection optics depends on two factors. The first is the ability to direct light with elements that will not routinely go out of alignment. The second is the ability to fix the location of the flow cell, allowing the distance between the flow channel, the illumination optics and collection optics to remain constant. This allows the illumination optics and the collection optics to also be located at fixed locations. It further allows the flow cell to be joined to the light collection optics in a way that minimizes loss of the collected light.

With respect to FIGS. **3** and **4**, the flow cell **41** is shown in front and side cross section respectively. The sheath flow tubes are not shown in these views. Generally sample delivery tube would be positioned such that the sample is introduced just before the neckdown region (the region

adjacent to the beginning of the flow channel) as shown in FIG. **3**. The two sheath flow delivery tubes provide sheath flow in an even, pulse free flow.

In FIG. **3**, the flow cell **41** is shown comprised of flow cell body **230**, cuvette **210**, nozzle key **214**, and flow cell base plate **220**. Flow cell body **230**, cuvette **210** and flow cell base plate **220** are joined together to form a single unit that may be secured in a fixed location by bolts extending through holes **209** onto a flow cytometer instrument (e.g. by bolting the flow cell onto a fixed position plate). The nozzle key **214** is not fixed and may be inserted into a location such that the nozzle is at the end of the flow cannel. The nozzle key could be subsequently removed, cleaned (e.g. sonicated) and reinserted. In addition, the fitting **233** containing the sample input tube also might be periodically removed and reattached. This allows the remaining portions of the flow cell to be in a fixed location within a flow cytometer.

The upper portion of the flow cell is the flow cell body **230**, which receives both the sheath flow tubes and the sample delivery tube **202**. The sheath flow liquid is delivered in tubes joined to fluid input body though ports **206**, **208**. The flow is delivered such that the sheath fluid surrounds a core of the sample stream as liquid passes through the flow channel **212**. The sheath fluid carries the core stream through a converging channel in flow cell body **230** and into the flow channel **212** in cuvette **210**.

The flow cell body **230** has an open top end through which the sample tube and oscillator are introduced. Inserted through the open top end is sample tube inlet fitting **233** and transducer plunger **232**. Plunger **232** is retained on boss **202** on flow cell body **230**. A tube (not shown) held by fitting **233** introduces a sample liquid through a passage in fitting **233**. This passage is joined to sample delivery tube **202** such that liquid flows through the passage, into sample delivery tube **202** and into a passage within the flow cell body. The sample delivery tube **202** terminates at an open end proximate to the flow channel **212** that extends through cuvette **210**. At this location the sample flowing through sample delivery tube **202** is surrounded by sheath flow fluid, forming the sample into a core in the flow stream as the stream moves through flow channel **212**.

The flow stream flows through flow channel **212** in cuvette **210** and exits at nozzle **216**. The length of flow channel H**1** is sufficiently long to ensure fully developed flow in the optical analysis region H**2** under all operating conditions. In the illustrated system a length of 8–15 mm is sufficient for a fully developed flow. Sidewalls **213** extend about three sides of nozzle key **214**, allowing registration of the nozzle key **214** in place. At the point of exit, the sample stream flow velocity increases as the sample exits the narrower nozzle opening.

Nozzle **216** is mounted on nozzle key **214**, positioned at a registered location at the end of cuvette **210**. The bottom side of nozzle key **214** rests on flow cell base platform **222** on flow cell base plate **220**. H**1** indicates a height of the cuvette between the flow cell body **230** and the nozzle key **214**. This is the location where illumination beams are directed through flow channel **212**. Close tolerances between the nozzle and the registration features insure that the direction of the stream does not change after a nozzle has been removed and replaced by the user.

FIG. **5** shows a detail of the nozzle key **214** and cuvette **210**. Nozzle key **214** has a nozzle key card **213** affixed to the top surface of the nozzle key **214**. The nozzle **216** is positioned on nozzle key card **213** on the nozzle key **214** such that the nozzle **216** is positioned at a selected location in the cross section of flow channel **212** when nozzle key **214** is inserted into cuvette **210**. When nozzle key is inserted into the registered position, nozzle key **214** is held between flow cell base platform **222** and the top surface of cuvette. The stream generated by flow through nozzle **216** flows into passage **218**.

The detail of the nozzle is shown in FIG. **7**. Cuvette **210** is shown having a flow channel **212**. At a terminus of flow channel **212** nozzle **216** is positioned. Nozzle **216** is shaped like a truncated funnel, producing a more stable flow stream. On nozzle key card **273**, an annular groove **261** holds an o-ring **260**. O-ring **260** seals nozzle key card **273** to cuvette **210** when nozzle key **214** is inserted in its registered position.

With reference to FIG. **3**, the stream in air which flows from nozzle **216** then passes through passage **218**. The sample could be collected here or the stream could be separated into droplets, allowing subsequent charging and sorting of droplets. To generate droplets, a drop drive piston **240** may be used. Signals for the power and operation of the drop drive piston **240** may be sent through transducer electrical terminal **235**. The electronic signal is sent to drop drive piezo element **234** held in transducer plunger **232**. Drop drive piezo element **234** oscillates drop drive piston **240**, sending oscillating pressure waves through the incompressible sheath flow fluid.

Previous systems have used vibration (as from a piezoelectric crystal) of the nozzle cuvette, or flow cell to generate droplets. The droplets generated are separated by the wavelength of the vibration. This allows division of the flow into individual droplets for sorting. However, the vibration of either the nozzle or the entire flow cell could have negative effects on the consistency of illumination and light collection if the vibration causes the relative distance from the flow channel and the illumination focus or light collection focus to change. This effect is more pronounced in stream-in-air optical interrogation. In the present invention, droplet generation originates from a displacement type oscillator near the source of the sheath flow. It has been found that the pressure waves are transmitted through the largely incompressible flow fluid and effectively generate the desired droplets.

With respect to FIG. **4**, the side cross-section shows detail of the key nozzle **214** as it is secured in place. In the side view, key **214** is shown having nozzle key grip **270**. Nozzle key **214** has a passage **218** defined by surface **254** and surface **256**. Nozzle key plunger **281** biases the nozzle against the sidewall of the cuvette, holding the nozzle in a registered position. The detail of the nozzle section shown in FIG. **6** shows the insertion of the nozzle between cuvette **210** and flow cell base platform **222**, holding nozzle key **214** in a position such that nozzle **216** is registered against the terminus of channel **212** extending through cuvette **210**, by the cell base platform **222**. Nozzle key plunger **281** provides a biasing pressure to retain nozzle key **214** in position. Shoulder **311** on nozzle key **214** is appressed against a surface of cuvette **210** when the nozzle key is fully inserted. This positions the nozzle at a registered position. The nozzle is prevented from being inserted too far, preventing damage to back wall **254**.

The details of the nozzle key are shown in FIGS. **9** and **10**. In FIG. **9**, nozzle key **214** is shown with nozzle **216** positioned on nozzle key card **273**. Label **271** affixed to the bottom of the card allows identification of the specific nozzle card used. Nozzle key plunger **281** provides a biasing force of the nozzle key **214** against the side walls of cuvette **210**. Nozzle key grip **270** allows a user to grip nozzle key **214** and remove it from cuvette **210**. In this way if the nozzle

were to become clogged, the nozzle could be simply removed, cleaned (e.g. sonicated) and replaced.

In FIG. **10**, the nozzle key is shown in exploded view. The nozzle key plunger **281** is inserted through the nozzle key **214**. Spring **282**, retained within nozzle key **214** by nozzle key spring plug **283**, provides a biasing force on plunger **281**. Plug **283** is retained on plug retainer **284**.

Nozzle key card is affixed on the top of nozzle **214**. O-ring **260** fits into groove **261** to provide a sealing force of the key card **273** to the cuvette when nozzle key **214** is inserted into the cuvette.

The use of a cuvette for the optical analysis of the stream allows for a lower excitation power requirements and greater efficiency of the collection optics. As opposed to analysis in a stream in air, the cuvette presents a stationary target with a flat interface for the incoming laser light from the illumination optics. Therefore, less light is lost to reflection and refraction. Because less light is lost, lower laser power is required. These features also make the collection of light more efficient. Less light is lost due to refraction of light from the stream to the light collection optics. In addition, the material transition from the stream to the collection optics can avoid the transition from liquid to air, with the attendant high index of refraction eliminated.

As noted in respect to FIGS. **1** and **2**, the use of the nozzle that may be inserted into a registered position allows fixing the position of the flow cell, illumination optics and the light collection optics. One advantage to this configuration is the elimination of wear and tear on the flow cell. When the flow cell is removed, it is possible that the surfaces through which light pass on the flow cell could become scratched or marred such that light collection or transmission to or from the illumination channel is altered. This is mitigated by fixing the flow cell in place and not requiring the flow cell to be moved or manipulated.

As noted, each material through which light passes will have a characteristic index of refraction. Light will be refracted when it passes from a medium having a first index of refraction to a medium having a second index of refraction. A major problem encountered in prior systems that optically analyze in a stream in air is the high index of refraction between the stream of liquid and air. This, coupled with illumination light losses due to the gross cylindrical nature of the stream in air, requires higher excitation power than is required in a cuvette system.

In a cuvette system, losses to refraction also occur in the transition from the cuvette to air material transition as emitted fluorescence moves from the cuvette, into air and subsequently into the collection lens. Fluorescence excited in a liquid moving through the flow channel is collected by a collection lens on one side of the flow channel. If the flow cell is fixed in location, the light collection optics may be physically joined to the cuvette. This reduces the refraction at the material transition from the cuvette to the collection lens.

With reference to FIGS. **12** and **14**, the cuvette **210** is joined to the emission collection lens in housing **50**. The nozzle key **214** is inserted into position such that the nozzle is positioned at the end of the flow channel. This is shown in FIG. **13**. In this cross sectional view, the flow channel **212** is shown extending through the cuvette **210**. The cuvette **210** is linked to an initial optical element **52** by a gel **290**. Light is collected by lenses in housing chamber **51**. One such collection lens is disclosed in U.S. patent application Ser. No. 09/934,741 entitled "Flow Cytometry Lens Systems".

Nozzle key **214** is inserted and registered against cuvette **210**. In one direction this registration is effected by biasing the key by nozzle plunger **281**, holding the nozzle card in position laterally.

The sidewalls **213** of the cuvette **210** extend below the exit plane of the cuvette (i.e. the plane containing the exit of the end of the flow channel). This allows for a larger numerical aperture for the collection of emitted fluorescence and for forward scatter. In addition the lower sidewalls **213** permit a lower entrance point for the laser beams, enabling the closest possible location of the optical interrogation region to the nozzle. In one embodiment the optical interrogation is 700 nm from the nozzle opening. This configuration ensures minimization of the delay time and least time delay error between the lowest laser illumination of the stream (target detection) and the droplet charging.

In prior flow cytometers in which optical analysis of a sample occurs in a cuvette channel, the cuvette would be of a block shape and the sidewalls would terminate at a bottom surface of the cuvette. In this configuration the illumination must occur a significant distance from the bottom of the cuvette, in order for efficient light collection of emitted fluorescence. If illumination occurs too close to the bottom of the cuvette, much of the potentially collectable fluorescent light will be lost from the bottom of the cuvette, which would refract the light away from the collection lens. To avoid this problem, the light collection would occur a significant distance from the bottom of the cuvette and thus a significant distance from the end of the flow channel. This may be acceptable for non-sorting applications, but for sorting applications the separation distance of the detection of targets and the nozzle is critical for determining drop delay and properly charging and deflecting droplets of interest. It is also critical towards avoiding time delay errors, which reduce sorting performance.

In addition to the lower sidewalls, the geometry of the channel also allows for more efficient illumination and light collection. In the present illustration, a rectangular cross sectional channel is used. The shorter side of the channel faces the illumination light and the longer side of the channel faces the collection lens. This allows collection from the area of the longer side of the channel. This presents a higher numerical aperture for collection. In FIG. **8** this is indicated by angle α. Collection from a higher numerical aperture allows more efficient collection of emitted light and greater sensitivity. This greater sensitivity enables use of lower power lasers. In addition, this wide viewing window allows keeping the cross-sectional area relatively small. This reduces the volumetric consumption of sheath flow required for the system.

The system shown in FIGS. **1** and **2** would be contained in a housing (not shown). This housing would prevent light from the area surrounding the system from entering the system.

Sorting droplets requires precise coordination of the detection of a target of interest, encapsulation of the target into a droplet during droplet formation, charging the droplet and sorting the droplet by passing the charged droplet containing the target of interest between two charged deflection plates. The flow of fluid into the flow cell is kept pulse free so that the perturbations of the fluid are minimized. This allows the general condition of directional stream in air stability.

It is desirable to have the flow stream break up into droplets in a predictable manner. In a sorting flow cytometer, the drop drive causes a leading order effect in which the flow stream-in-air, after flowing from the nozzle, breaks into a

train of large droplets having a characteristic diameter of the same order of magnitude as the jet diameter, as shown in FIG. **15***a*. Due to the nonlinearity of the fluid dynamics characteristic of flow cytometry, smaller droplets **306***a* typically form between the larger "parent" droplets **304***a*. The smaller droplets are referred to as "satellite droplets". It is advantageous to have a stream condition in which no satellites form, or in which the satellite droplets that do form quickly merge into the parent droplets. The satellite droplets are significantly smaller, and hence have lower masses, than the parent droplets.

During particle sorting, sorting is accomplished by selectively charging droplets. The droplets then pass through an electric field that deflects the path of the charged droplets so that the charged droplets are deflected from the rest of the droplet stream. The deflected droplets are deflected into a separate collection container for later use or analysis. The required magnitudes of both the droplet charging and electric field potentials of the charging plates are selected to provide the needed deflection of the parent droplets. The smaller satellite droplets that are deflected by the charged deflection plates may be so light that the particles are directed out of the flow stream and onto the charging plates. The resulting wetting of the charging plates may adversely affect system performance and require interruption of the use of the system to dry and clean the deflection plates. In addition, the deflection of satellite droplets could present biohazard risks, especially if the satellite droplets form aerosol droplets that remain suspended in the air.

In the prior systems, favorable satellite conditions were achieved through trial and error. A user could make ad hoc adjustments to the drop-drive amplitude, drop-drive frequency, and sheath pressure until a favorable satellite droplet conditions are achieved (i.e. satellites quickly merged with parent droplets). This is largely guesswork, requiring a knowledgeable user and some time. Optical systems that monitor droplet formation are required to determine that the satellite droplets are merging with the parent droplets.

Theoretically, a perfectly symmetric jet excited near its spontaneous drop frequency will break into a droplet chain whose satellite droplets never merge with the parent droplets. A portion of the drop drive energy cascades into a secondary satellite droplet formation harmonic, in phase with the fundamental droplet formation frequency. Some experiments have shown that one method to control the satellite formation is to add a phased, higher-harmonic component to the drop drive vibration or pulsation to alter or cancel satellite development. (see Chaudhary, K C, and Redekopp, L G, "The nonlinear Capillary Instability of a Liquid Jet. Parts 1–3" J. Fluid Mech., Vol. 96 (1980a–c)).

Location of the nozzle in a precise location in relation to the flow channel allows creation of a repeatable and favorable satellite droplet merging conditions. One embodiment of the present invention uses the nozzle location to ensure more optimal satellite droplet merging. With reference to FIG. **8**, a detail of the cross section of the cuvette **210** shows the flow channel **212** with a circle indicating the nozzle opening **294**. The center **292** of nozzle opening **294** is positioned off center from the cross sectional center of flow channel **212**. A small lateral adjustment of the centering of the nozzle in the flow channel provides a more favorable condition for merger of the satellite droplets with the parent droplets.

In one embodiment of the invention the nozzle is purposefully misaligned with respect to the center of the flow channel. This misalignment may be achieved by machining 0.001 inch from the nozzle registration feature that locates the nozzle in the long dimension of the cuvette channel (e.g. the sides of the nozzle card). The present system allows precise location of an insertable nozzle card. The ability to precisely and repeatably locate the nozzle allows design of the nozzle card such that the nozzle is off from the flow channel center.

The nozzle location is fully constrained by hard features on the nozzle that register directly against the cuvette (or flow cell) such that the nozzle orientation is always fixed in three dimensions with respect to the exit of the cuvette channel. The registration of the nozzle in this manner minimizes tolerance stack up. The limiting factors of this approach are the manufacturing tolerances associated with manufacturing the nozzle and the cuvette. State of the art manufacturing procedures allow nozzle location to +/−0.0012" (+/−30.48 μm) in the plane of the channel. Given these manufacturing tolerances, nozzle-to-nozzle stream performance has proven to be consistent. A given nozzle will always register against a given cuvette in the same manner, ensuring a consistent stream direction and droplet formation pattern.

As noted, the theoretical model of droplet formation indicates that an axisymetric flow stream excited by a fundamental frequency in the range of the spontaneous droplet frequency will break up into a droplet chain in which the satellite droplets never merge with the parent droplets. This implies that deviation from perfect axisymetry could allow for more optimal satellite droplet merging conditions.

In the present illustration, the nozzle is adjusted laterally in the long dimension. This is generally preferred, as it produces the smallest variation in the path for particles within the flow stream. This minimizes the difficulty in timing the delay between detection in the channel and charging and sorting droplets after the droplets have passed through the nozzle. It is also possible to have the displacement in the shorter dimension or displace the centering in both the long and the sort dimensions (i.e. displace on a diagonal from the cross sectional center of the stream).

With reference to FIG. **15***a*, an image of the stream shows droplet formation in a system in which the center of the nozzle opening is closely aligned with the center of the flow channel. At the top, the stream has begun to break into individual droplets. Height A marks the distance from the formation of the first droplet broken from the flow stream to the area where the satellite droplets have combined into the parent droplets. It would be preferred that droplet deflection not occur before this location. Parent droplet **304***a* and satellite droplet **306***a* are identified close to the location of droplet formation at the top of height A. A merging parent and satellite droplet **305***a* are identified close to the bottom of height A. Eight or more droplet wavelengths are required before the parent droplet and the satellite droplets have merged.

With reference to FIG. **15***b*, an image of the droplet formation pattern in which the nozzle has been misaligned is shown. This is the misalignment shown in FIG. **8**, in which a rectangular flow channel is used. The dimensions of the flow channel in this embodiment is 250 um by 160 um. The nozzle is deliberately shifted 25.4 um off of the centerline of the cuvette in the long axis of the cuvette. In FIG. **15***b*, height B is a height from the location of a droplet formation to a location where the droplets do not show any satellite droplets. Satellite droplet **306***b*, shown just after droplet break-off point, and parent droplet **304***b* are shown. A merging parent droplet with a satellite droplet **305***b* is just below. In three droplet wavelengths the parent droplets have merged with the satellite droplets.

It will be readily appreciated by a person of ordinary skill in the art that a number of modifications to the present invention are possible. The nozzle opening, as presently illustrated, is a truncated tapered cone. A card is affixed to a nozzle key such that the nozzle is in a precisely registered location when the card is inserted into the flow cell. The nozzle may take many shapes and geometries. For example, an elongate nozzle opening may be preferable in some applications to a round opening. The nozzle may be a lengthened truncated cone, extending downstream from the flow stream direction. In this way the transition from the width of the flow channel to the nozzle may be made less abrupt, and could be a continuous graded tapering to the nozzle opening. In addition, the substrate on which the nozzle opening is formed may use a number of different designs.

A number of the elements of the present invention could be used independently or in a number of different combinations. The fixed flow cell with nozzle key may be adapted into present systems in which the illumination optics and light detection optics may be aligned. Alternatively, the fixed flow cell may be used with either fixed location light collection optics (e.g. fluorescent light collector and light scatter collectors) or a fixed location illumination optics. Other optical elements within such systems would still require routine adjustment. Such systems would retain the advantage of having a fixed position flow cell that does not require to be removed or adjusted. In some embodiments, the cuvette may not be physically coupled to the light collection optics. Such embodiments could have all of the attendant optics (illumination optics, light collection optics, and scatter detection optics) in a fixed location or have some or all of these elements mounted on an adjusting mounts.

The droplet drive may be generated by an oscillator within the flow cell, allowing transfer of the oscillating pulse to the largely incompressible fluid. Alternatively, the droplet drive may be generated by more conventional means, such as vibrating the nozzle, cuvette or vibrating the entire flowcell assembly. Additionally, the droplet drive may be generated by acoustic vibration of the stream in air.

In the illustrated embodiment, the flow cell body is joined to a rectangular cuvette and the nozzle is inserted at the terminus of the cuvette. The term "cuvette" in various embodiments, is the flow cell section through which the channel extends. This may be a separate component joined to a flow cell body. Alternatively, the cuvette may be part of the flow cell body, which may be manufactured as a single part.

In the preferred embodiment, the nozzle is on a substrate that is inserted into a fixed position where it is registered against surfaces to hold the nozzle in a three-dimensional position such that the nozzle cannot angularly rotate. This allows the cuvette and flow to be in a fixed position. Because the channel is in a fixed position, the illumination optics and light collection optics may also be in a fixed position. In addition, the cuvette may be physically joined to the collection lens. It is also foreseen that a flow cell could be made in which the nozzle is a fixed part of the cuvette and the cuvette and nozzle are removed and inserted together. Precision guides could be used to precisely position the cuvette at the required location needed for alignment with illumination optics. The cuvette could be removed, sonicated or otherwise treated to clear a clog in the nozzle or flow channel, and replaced into a precise position. Because the droplet generator is in the flow cell body, the cuvette and nozzle could be removed and reinserted without having to reconnect to a vibration generator.

The invention was illustrated in a system in which multiple lasers are directed into the system using optical fibers. The beams are redirected and shaped using refractive optics. It is envisioned that a single laser or any number of lasers may be used. The lasers could be positioned on the platform and held in a fixed position (e.g. using diode lasers). It is also possible to employ non-laser light sources such as arc lamps. In place of the refractive optics, the conventional use of steering mirrors and dichroic mirrors could be used to direct and shape the illumination beams. In addition, spatial filters, long or short pass filters, apertures or other optics may be employed to block stray light and reduce transmission of undesired wavelengths. Systems employing conventional optics are disclosed in the references cited herein, which are collectively incorporated by reference.

The flow channel in the present illustration is rectangular. In other embodiments, a round flow channel or other geometries are envisioned. In the illustrated embodiment, nozzle is on a removable key. However, the nozzle could also be precision aligned and affixed (by sonic welding, adhesive attachment, etc.) to the end of the flow channel. Such a system would require some means of back-flushing the nozzle to clear clogs.

The illustrated system discloses the use of the flow cell in a sorting flow cytometer. While the present invention provides numerous advantages when employed in a sorting cytometer, it is also considered that disclosed technology could be used in non-sorting analytical cytometers.

The interior of the flow cell could be coated to prevent adhesion of cells, cell fragments or other compounds. Such a treatment would be selected to not be affected by the flow fluid. The flow cell is not removed but may be selectively flushed. The sheath flow system may be designed to allow for system flushing. If fluid is introduced through a first sheath flow port and removed through a second port, flow conditions would direct the flow in a vortex through the flow cell interior, washing all elements.

Because the nozzle is smaller than the flow channel, any clog is likely to occur in the nozzle, which may be removed and cleaned. If the channel clogs, the nozzle may be removed and the channel cleared with back pressure.

The present invention allows development of a system in which the user does not have to perform any routine optical alignment procedure. The user could remove and replace the nozzle without further alignment of the stream direction or optics. The analysis is effected in a cuvette, with the attendant sensitivity allowed by analysis in a cuvette. Sorting occurs in the steam in air, allowing sorting in the conventional manner. The relatively low velocity of the core stream in the analysis region is advantageous for analysis of targets. The stream is then accelerated by the nozzle, to allow for high speed sorting. These advantages present a considerable cost savings due to both time saved as well as skill required to use the system. This system also significantly improves the depth and sensitivity of analysis and sorting performance.

What is claimed is:

1. A flow cell for use with a flow cytometer comprising:

a flow cell body;

a sample delivery tube extending into said flow cell body;

at least one sheath flow port on said flow cell body, said at least one port allowing introduction of a flow of sheath flow liquid through said flow cell body;

a cuvette having flat sides and a rectanaular cross-section, joined to the flow cell body;

a channel extending through the cuvette, said channel comprising an initial end and a terminal end, wherein

liquid from said sample delivery tube and said at least one sheath flow port flows into said initial end of said channel; and

a removable nozzle at said terminal end of said channel through which said liquid from said sample delivery tube and said at least one sheath flow port flows out of said channel, wherein said removable nozzle is positioned on a removable nozzle key having hard planar surfaces on a top and on at least two sides, said nozzle held in a registered position on said flow cell, said registered position at a defined three-dimensional position and at a registered rotational orientation, wherein said nozzle is held in said registered position by contact between said hard planar surfaces of said nozzle key and said cuvette.

2. The flow cell of claim **1**, further including a droplet generator associated with said flow cell, said droplet generator allowing production of droplets as a stream exits the nozzle.

3. The flow cell of claim **2**, wherein said droplet generator is located within said flow cell body such that droplet generation oscillations are imparted to flow fluid before said fluid enters the channel.

4. The flow cell of claim **3**, wherein said droplet generator includes a piston.

5. The flow cell of claim **2**, wherein said droplet generator is a vibrating element.

6. The flow cell of claim **5**, wherein said vibrating element vibrates one of a group consisting of the nozzle, the cuvette, and the flow cell body.

7. The flow cell of claim **1**, wherein said nozzle key includes a biasing spring on one side of said key.

8. The flow cell of claim **1**, wherein said nozzle key includes an o-ring surrounding the nozzle.

9. The flow cell of claim **1**, wherein said cuvette has optically transmissive sidewalls that extend downward from a location of said terminus of said channel.

10. The flow cell of claim **1**, wherein said channel baa a rectangular cross section.

11. The flow cell of claim **1**, wherein said channel has a length sufficiently long so that liquid flowing through said channel has fully developed flow when said fluid reaches an illumination location on said channel.

12. The flow cell of claim **1**, wherein when said nozzle is inserted into said registered location, a nozzle opening of to nozzle is off center from a centered position within the channel.

13. A flow cytometer system comprising:

(a) a flow cell, wherein said flow cell comprises

a flow cell body;

a sample delivery tube extending into said flow cell body;

at least one sheath flow port on said flow cell body, said at least one port allowing introduction of a flow of sheath flow liquid through said flow cell body;

a cuvette having flat sides and a rectangular cross-section, joined to the flow cell body;

a channel extending through the cuvette, said channel comprising an initial end and a terminal end, said channel joined to said flow cell body such tat liquid from said sample delivery tube and said at least one sheath flow port flows into said initial end of said channel; and

a removable nozzle at said terminal end of said channel through which said liquid from said sample delivery tube and said at least one sheath flow port flows out of said channel, wherein said removable nozzle is positioned on a removable nozzle key having hard planar surfaces on a top and on at least two sides said nozzle held in a registered position on said flow cell, said registered position at a defined three-dimensional position and at a registered rotational orientation, wherein said nozzle is held in said registered position by contact between said hard planar surfaces of said nozzle key and said cuvette;

(b) illumination optics which focus and direct illumination light into said flow cell at an illumination region of said flow cell; and

(c) light collection optics which collect light produced from targets in the channel of the flow cell and transmit collected light to detection optics.

14. The system of claim **13**, further including a droplet generator associated with said flow cell, said droplet generator allowing production of droplets as a stream exits the nozzle.

15. The system of claim **14**, wherein said droplet generator is located within said flow cell body such that droplet generation oscillations are imparted to flow fluid before said fluid enters the channel.

16. The system of claim **15**, wherein said droplet generator includes a piston.

17. The system of claim **14**, wherein said droplet generator is a vibrating element.

18. The system of claim **17**, wherein said vibrating element vibrates one of a group consisting of the nozzle, the cuvette, and the flow cell body.

19. The system of claim **13**, wherein said nozzle key includes a biasing spring on one side of said key.

20. The system of claim **13**, wherein said nozzle key includes en o-ring surrounding the nozzle.

21. The system of claim **13**, wherein said cuvette has optically transmissive sidewalls that extend downward from a location of said terminus of said channel.

22. The system of claim **13**, wherein said channel has a rectangular cross section.

23. The system of claim **13**, wherein said channel has a length sufficiently long so that liquid flowing through said channel has fully developed flow when said fluid reaches the illumination region.

24. The system of claim **13**, wherein when said nozzle is inserted into said registered location, a nozzle opening of the nozzle is off center from a centered position within the channel.

25. The system of claim **13**, further including alight transmissive coupling joining the cuvette with a light collection lens, said lens being an element of the light collection optics.

26. The system of claim **22**, wherein said channel is rectangular and has a shorter cross-sectional side and a longer cross-sectional side, wherein said shorter cross-sectional side faces a first direction in which light is directed by the illumination optics and the longer cross-sectional side faces a second direction from which emitted light is collected by the light collection optics.

* * * * *

EXHIBIT 4

US007787197B2

(12) **United States Patent**
Chen

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(45) **Date of Patent: Aug. 31, 2010**

(54) **BEAM-ADJUSTING OPTICS**

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See application file for complete search history.

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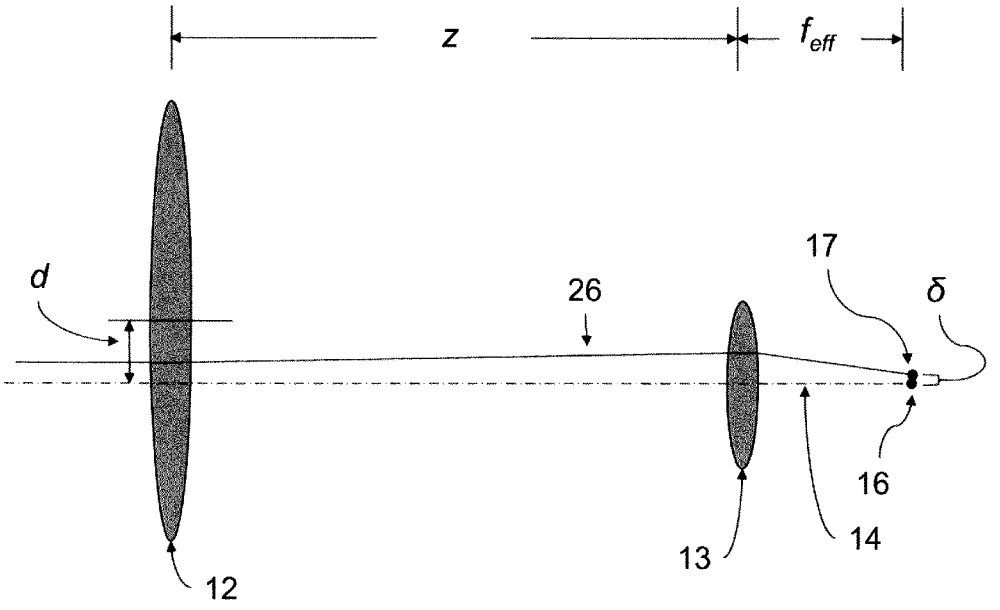
(57) **ABSTRACT**

The present invention provides an optical analyzer having illumination optics that include a light source, such as a laser or other source, adapted to emit a collimated, or approximately collimated, light beam, a focusing lens that focuses the beam onto a focus spot within a detection region, and beam-adjusting optics positioned in the light path between the light beam source and the focusing lens, which allow for precise positioning of the focus spot within the detection region. The beam-adjusting optics of the present invention comprise at least one movable focusing lens, mounted in a positioning device that allows repositioning of the lens in a plane perpendicular to the light path.

13 Claims, 4 Drawing Sheets



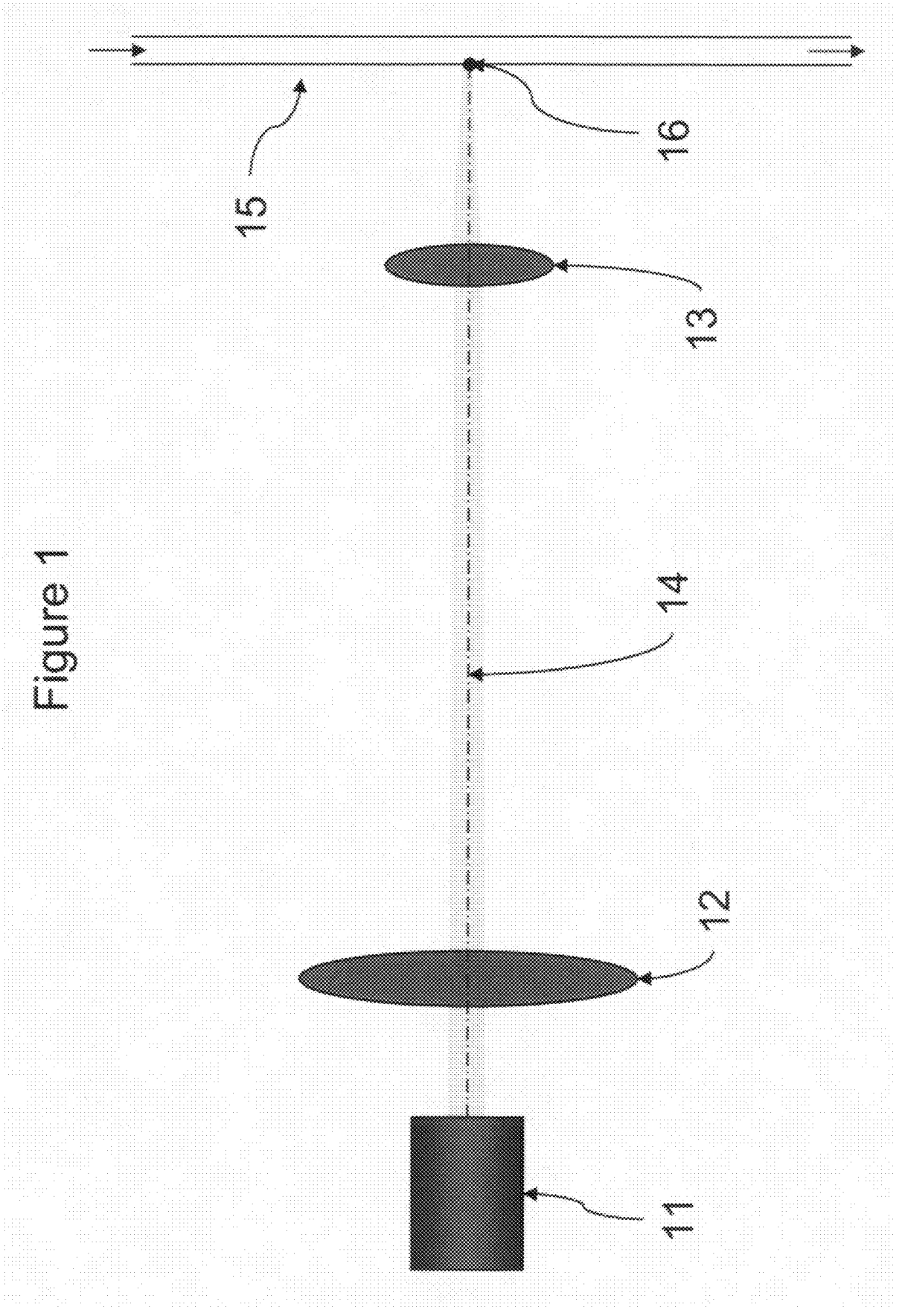


Figure 1

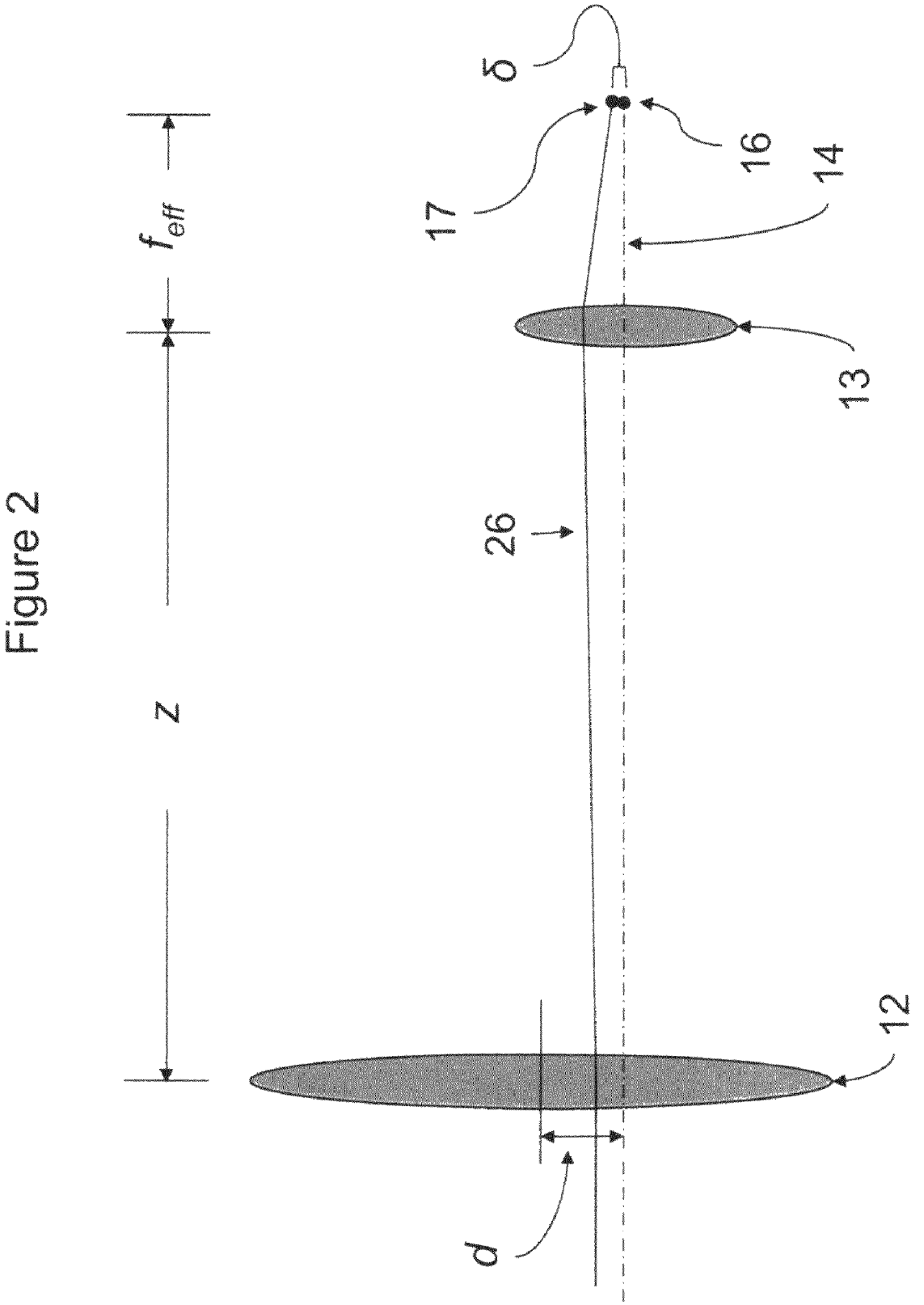


Figure 2

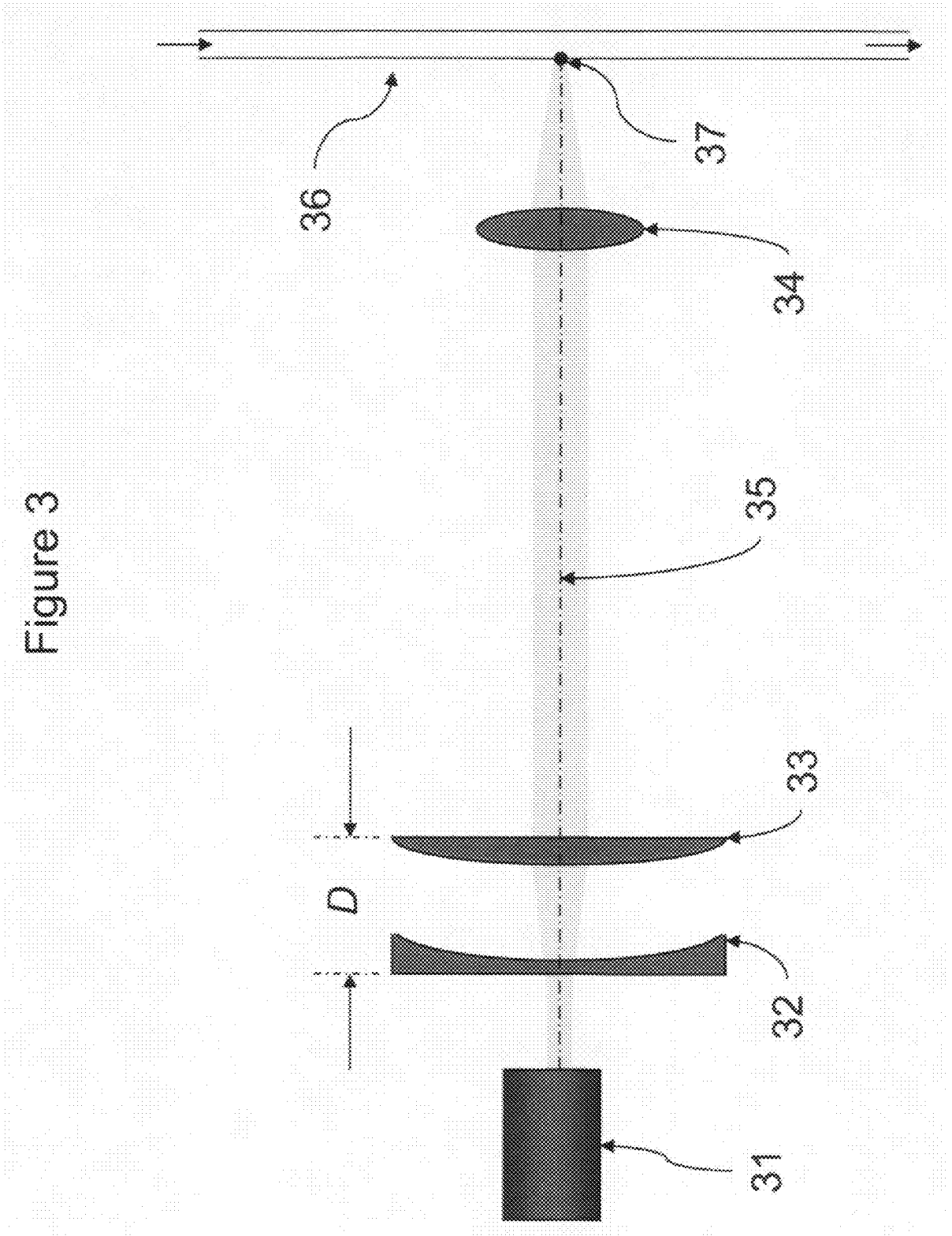


Figure 3

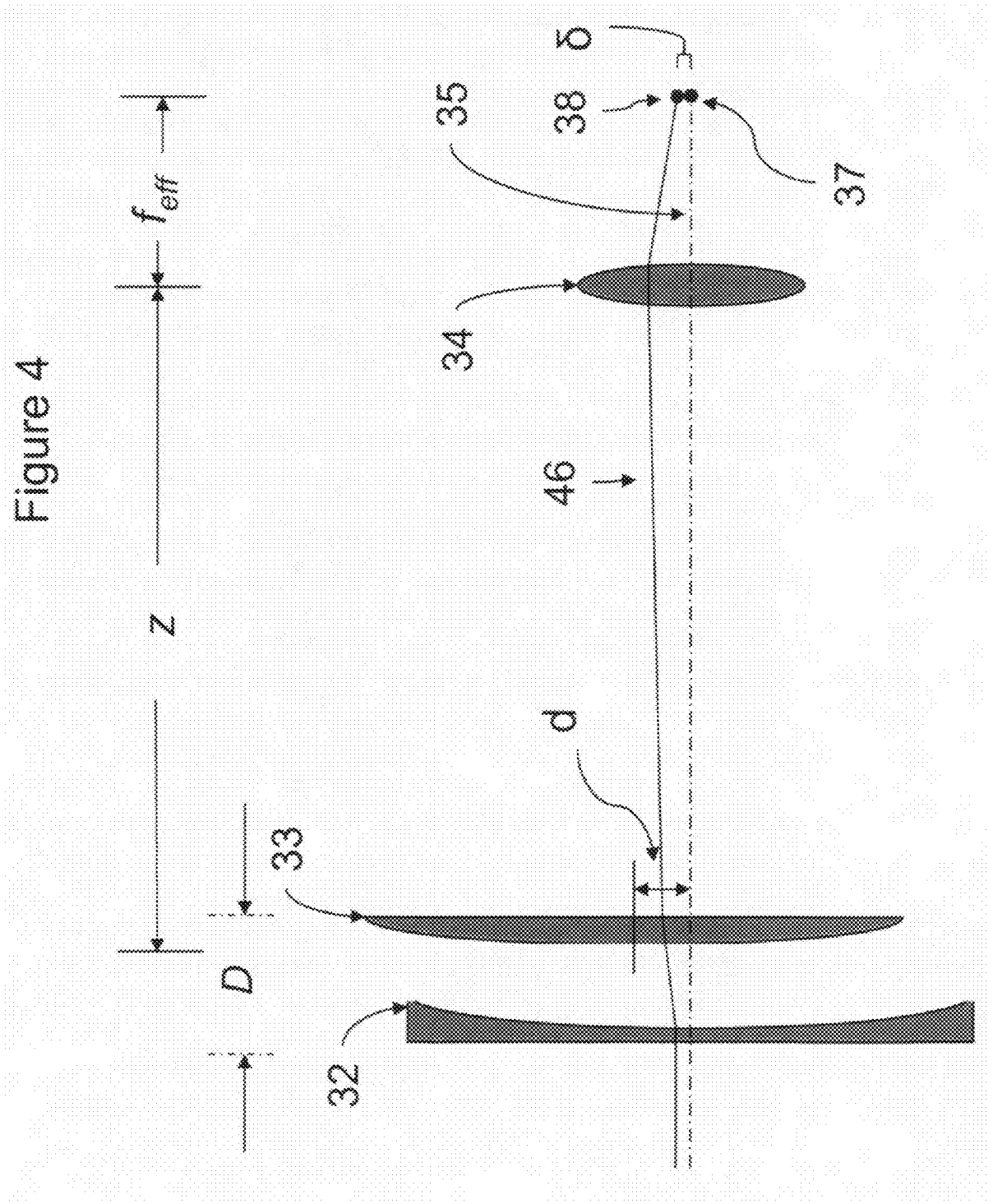


Figure 4
f_{eff}
z
D
32
33
34
35
37
38
46
d
b

BEAM-ADJUSTING OPTICS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. provisional application Ser. No. 60/993,758, filed Sep. 14, 2007, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the field of optics and, in particular, to laser optics, as used in optical analyzers.

2. Description of Related Art

Particle analyzers, such as flow and scanning cytometers, are well known analytical tools that enable the characterization of particles on the basis of optical parameters such as light scatter and fluorescence. In a flow cytometer, for example, particles, such as molecules, analyte-bound beads, or individual cells, in a fluid suspension are passed by a detection region in which the particles are exposed to an excitation light, typically from one or more lasers, and the light scattering and fluorescence properties of the particles are measured. Particles or components thereof typically are labeled with fluorescent dyes to facilitate detection, and a multiplicity of different particles or components may be simultaneously detected by using spectrally distinct fluorescent dyes to label the different particles or components. Typically, detection is carried out using a multiplicity of photodetectors, one for each distinct dye to be detected. Both flow and scanning cytometers are commercially available from, for example, BD Biosciences (San Jose, Calif.). A full description of flow cytometers is provided in Shapiro, 2003, Practical Flow Cytometry, 4th ed. (John Wiley and Sons, Inc. Hoboken, N.J.), and in the references cited therein, all incorporated herein by reference.

In a typical flow cytometer, the excitation light from a laser or other source is focused onto a focal spot to illuminate the core stream (the fluid stream containing the particles to be analyzed). Accurate focusing of the excitation light beam on the core stream is important for optimizing focal spot intensity and, thus, fluorescence sensitivity. Optimal performance is compromised if the focused light beam is not properly adjusted on the core stream, and flow cytometers typically include one or more devices for adjusting the positioning of the focused light beam on the core stream. Because a typical flow cytometer is designed to analyze biological cells or particles that are few microns in size, the precision of the light beam adjustment also needs to be in the micron range, thus requiring high resolution mechanical displacement devices. Conventional positioning methods typically employ expensive differential micrometers to position the light source itself or optical elements, such as mirrors or prisms.

U.S. Pat. No. 4,989,977 describes one device for the accurate adjustment of the focused excitation beam on the core stream. Repositioning of the focal point is achieved using a transparent glass plate located between the focusing lens and the core stream. The glass plate, when positioned at an angle to the beam path, displaces the focal point by refracting the beam. In a multi-laser instrument, the glass plate typically is positioned between the focusing lens and the core stream, and all beams in a multi-laser instrument are passed through the single plate. One disadvantage of this typical implementation is that independent adjustment of the focal spot of each laser is not easily implemented.

BRIEF SUMMARY OF THE INVENTION

The present invention provides illumination optics for use in an optical analyzer that includes a light source, such as a laser or other source, adapted to emit a collimated, or approximately collimated, beam, a focusing lens that focuses the beam onto a focus spot, and beam-adjusting optics positioned in the light path between the light beam source and the focusing lens, which allow for precise positioning of the focus spot of the focused light beam. The beam-adjusting optics of the present invention comprises at least one movable focusing lens, mounted in a positioning device that allows repositioning of the lens in a plane perpendicular to the light path. The size of the movable focusing lens will be sufficiently larger than the width of the collimated beam such that the beam passes through the movable lens when the lens is repositioned.

The present invention further provides an optical analyzer incorporating the illumination optics of the present invention, adapted to focus an illumination beam onto a sample analysis region. The optical analyzer will further comprise detection optics for measuring the light emitted from the analysis region. In a preferred embodiment, the optical analyzer is a flow cytometer, and the sample analysis region is a sample detection region in a fluid stream containing particles to be optically analyzed. Typically, the detection optics detect illumination light scattered by particles in the flow stream, as well as fluorescent light emitted by the particles after being excited by the illumination light.

In one embodiment, the beam-adjusting optics of the present invention comprise a movable beam-adjusting lens that is a long focal length lens, positioned in the optical path such that the optical axis of the lens is parallel to the optical path, wherein the width of the lens is sufficiently larger than the width of the excitation beam to allow for movement of the lens in a plane perpendicular to the optical path while remaining in the optical path.

Increasing the focal length of the movable beam-adjusting lens decreases the sensitivity of the focus spot positioning to changes in the position of the beam-adjusting lens, i.e., increasing the focal length of the movable beam-adjusting lens will decrease the displacement of the focus spot in the sample detection region for a given displacement of the beam-adjusting lens. The decreased sensitivity to movement of the movable beam-adjusting lens allows the use of less expensive, less precise lens positioning mechanisms, such as simple screw-type positioning systems, to obtain precise positioning control over the beam focus spot. As general guidance, the focal length of the movable beam-adjusting lens, minus the distance between the movable beam-adjusting lens and the focusing lens, preferably is at least two times as long as the focal length of the focusing lens, more preferably at least four times as long, and even more preferably, at least six times as long.

Typically, the long-focal length lens is a spherical lens, which allows adjustment of the beam focus spot along both axes perpendicular to the optical path. Depending on the application, it may be sufficient to provide adjustment of the focus spot in only one direction, in which case a cylindrical lens is suitable.

In another embodiment, the beam-adjusting optics of the present invention comprise a converging lens having a positive focal length (e.g., a convex lens) and a diverging lens having a negative focal length (e.g., a concave lens), located a short distance apart other along the optical path and positioned in the optical path such that the optical axis of each lens is parallel to the optical path. At least one of the converging

lens and diverging lens is mounted in a positioning device such that the lens can be moved in a plane perpendicular to the optical path, and functions as the beam-adjusting lens. The width of the beam-adjusting length lens is sufficiently larger than the width of the excitation beam to allow for movement of the lens in a plane perpendicular to the optical path while remaining in the optical path. The use of a converging lens along with a diverging lens enables significantly increasing the equivalent focal length of the beam-adjusting optics, which minimizes the effect of the lens pair on the effective focal length of the illumination optics, but which has minimal effect on the beam-adjusting property of the beam-adjusting lens.

In a preferred embodiment, the beam-adjusting optics of the present invention comprise a plano-concave lens and a plano-convex lens, located a short distance apart other along the optical path, positioned in the optical path such that the optical axis of each lens is parallel to the optical path, and oriented such that the concave and convex faces of the lenses are facing each other. At least one of the plano-concave lens and a plano-convex lens is mounted in a positioning device such that the lens can be moved in a plane perpendicular to the optical path, and functions as the beam-adjusting lens. The width of the beam-adjusting length lens is sufficiently larger than the width of the excitation beam to allow for movement of the lens in a plane perpendicular to the optical path while remaining in the optical path.

In a preferred embodiment, the plano-concave lens and the plano-convex lens are matched, i.e., the focal lengths of the lenses are of the same magnitude, but of opposite sign, and the distance between the lens is small, such that parallel light beams entering the beam adjustment optics will exit the beam adjustment optics almost parallel. In this embodiment, the equivalent focal length of the lens pair is much longer than the focal length of the individual lenses, and the lens pair has a negligible effect on the effective focal length of the illumination optics.

In a preferred embodiment, the optical analyzer of the present invention is a flow cytometer and the beam-adjusting optics are component of the illumination (excitation) optics, used to adjust the illumination light focused on the detection region of the flow stream. However, fine control over the focus spot of a illumination beam can be useful in a variety of applications, and the present invention will be generally useful in applications in which fine control over the focus spot of a illumination beam is useful. Other applications in which the illumination optics of the present invention may be useful include, for example, microscopy and laser scanning cytometry.

The beam-adjusting optics of the present invention are particularly suited for use in the illumination light optics of a multi-laser optical analyzer. As the beam adjustment optics can be located anywhere before the focusing lens, individual beam-adjusting optics can be used for each of the lasers in a multi-laser system, thus enabling independent adjustment of the focal spot for each of the lasers.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. **1** shows a schematic representation of an embodiment of the illumination optics of the present invention in which the beam-adjusting optics consist of a single long focal length lens.

FIG. **2** shows a schematic representation of the path of a light ray through the illumination optics shown in FIG. **1**.

FIG. **3** shows a schematic representation of an embodiment of the illumination optics of the present invention in which the beam-adjusting optics consist of a plano-concave lens and a plano-convex lens.

FIG. **4** shows a schematic representation of the path of a light ray through the illumination optics shown in FIG. **3**.

The figures depict schematic representation of optical systems and are not drawn to scale. The convention in all the figures is that light propagates from left to right through the optical system.

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are provided for clarity. Unless otherwise indicated, all terms are used as is common in the art. All reference cited herein, both supra and infra, are incorporated herein by reference.

As used herein, the "equivalent focal length" or "combined focal length" of a compound optical system refers to the focal length of a compound optical system, given as if it were a single optical element. The equivalent focal length is the distance from the secondary principle point of the compound optical system to the focal point. The equivalent focal length, f_{eq}, for a combination of two components is related to the focal lengths of the two components by the following equation:

$$f_{eq} = \frac{f_1 \cdot f_2}{f_1 + f_2 - d}, \tag{1}$$

wherein f_1 and f_2 are the focal lengths of the individual components and the d is the distance between the components. The equivalent focal length of a optical system containing more that two components can be calculated by first calculating the equivalent focal length for the first two components, then performing the same calculation using the equivalent focal length for this combination and the focal length of the next lens. This is continued until all lenses in the system are accounted for.

As used herein, the "effective focal length" of a compound optical system refers to the focal length at which an optical system seems to be working in a given situation. The effective focal length is the distance from the secondary principle point of the second (or final) lens to the focal point. The effective focal length for a combination of two components is related to the focal lengths of the two components by the following equation:

$$f_{eff} = \frac{f_2 \cdot (f_1 - d)}{f_1 + f_2 - d}, \tag{2}$$

wherein f_1 and f_2 are the focal lengths of the individual components and the d is the distance between the components, or, equivalently, by the following equation:

$$f_{eff} = \frac{f_2}{1 + \frac{f_2}{(f_1 - d)}}. \tag{3}$$

In many embodiments of the invention, the beam-adjusting optics will consist of one or more "thin lenses". A thin lens is

a lens with a thickness (distance along the optical axis between the two surfaces of the lens) that is negligible compared to the focal length of the lens. The optical properties may be approximated using a "thin-lens approximation" in which the thickness of the lens is ignored. Under a thin-lens approximation in which the thickness of the lens is assumed to be zero, the primary and secondary principle points lie in the plane of the lens, and the effective focal length is the distance from the plane of the final lens to the focal point.

Illumination Optics

The illumination optics (also referred to as excitation optics) of the present invention include a light source, such as a laser or other source, adapted to emit a collimated, or approximately collimated, beam, a focusing lens that focuses the beam onto a focus spot, and beam-adjusting optics positioned in the light path between the light beam source and the focusing lens, which allow for precise positioning of the focus spot of the focused light beam. The beam-adjusting optics of the present invention comprises at least one movable focusing lens, mounted in a positioning device that allows repositioning of the lens in a plane perpendicular to the light path. The size of the movable focusing lens will be sufficiently larger than the width of the collimated beam such that the beam passes through the movable lens when the lens is repositioned.

Light Source

Light sources suitable for use in optical analyzers are well known in the art and commercially available from a large number of sources. Example include lasers, arc lamps, and light emitting diodes. For use in the present invention, the emitted light beam should be collimated or approximately collimated. It will be understood that the light source may include collimating optics. A discussion of light sources for use in flow cytometry can be found in, for example, Shapiro, 2003, Practical Flow Cytometry, 4th ed. (John Wiley and Sons, Inc. Hoboken, N.J.), incorporated herein by reference.

Focusing Lens

Focusing lenses are a standard elements well-known in the art and commercially available from a large number of sources. The particular lens design used in the present invention will be application dependent, and one of skill in the art will be able to select a suitable focusing lens routinely following the guidance provided herein. A discussion of focusing lenses for use in flow cytometry can be found in, for example, Shapiro, 2003, Practical Flow Cytometry, 4th ed. (John Wiley and Sons, Inc. Hoboken, N.J.), incorporated herein by reference. Typically, lenses are fabricated of fused silica for maximum light transmission, although any suitable material may be used.

As exemplified herein, a focusing lens typically consists of a single element. However, more complex focusing optics can be used. For example, crossed cylindrical lenses having different focal lengths have been used in flow cytometers to focus a laser beam to an elliptical spot on the sample stream. The focusing optics may additional comprise other elements, such as beam shaping optics, such as described in U.S. Pat. No. 4,498,766 and U.S. Patent Application Publication No. 2006-0256335, both incorporated herein by reference.

Description Based on the Figures

While this invention is satisfied by embodiments in many different forms, shown in the drawings and described herein in detail are preferred embodiments of the invention, with the understanding that the present disclosure is to be considered as exemplary of the principles of the invention and is not intended to limit the invention to the embodiments illustrated.

Single-Lens Beam-Adjusting Optics

FIG. **1** shows a schematic representation of an embodiment of the illumination optics of the present invention in which the beam-adjusting optics consist of a long focal length lens **12** having focal length f_{12}. Light source **11**, which typically is a laser, emits an essentially collimated beam having an optical path **14**, which is focused to focal spot **16** by focusing lens **13** having focal length f_{13}. Focal spot **16** corresponds to the detection region in a sample stream **15** containing particles to be optically analyzed. Lens **12** is mounted such that it can be moved in a plane perpendicular to the optical path, such as by using a mechanical positioning system (not shown). In FIG. **1**, lens **12** is positioned such that optical path **14** passes through the center of the lens. In this configuration, the focal spot **16** of the illumination optics is not displaced by the beam-adjusting optics from the optical path **14**.

FIG. **2** shows a schematic representation of the illumination optics shown in FIG. **1**, wherein lens **12** has been displaced by a distance d from the optical path in a plane perpendicular to the optical path. The path of an arbitrary light ray **26** through the beam illumination optics is shown. The optical effect of displacing lens **12** a distance d in a plane perpendicular to the optical path is to move the focal spot to new focal spot **17**, displaced from focal spot **16** by a distance δ.

The optical effect of lens **12** on the illumination optics, relative to illumination optics having only focusing lens **13**, is two-fold. First, the addition of lens **12** modifies the focal length of the illumination optics. Second, displacement of lens **12** in a plane perpendicular to the optical path displaces the focal point of the illumination optics.

From equation (3), above, the effective focal length, f_{eff}, of the illumination optics with lens **12** is

$$f_{eff} = \frac{f_{13}}{1 + \frac{f_{13}}{f_{12} - z}}, \tag{4}$$

wherein f_{12} and f_{13} are the focal lengths of lens **12** and lens **13**, respectively, and z is the distance between lens **12** and lens **13**.

The transverse displacement of the focal point, δ, resulting from displacing lens **12** a distance d in a plane perpendicular to the optical path is

$$\delta = \frac{d}{1 + \frac{f_{12} - z}{f_{13}}}. \tag{5}$$

Thus, the beam-adjusting lens allows for a lateral displacement of the focal spot of the illumination optics that is proportional to the displacement of the beam-adjusting lens in a plane perpendicular to the optical-axis. In a preferred embodiment, lens **12** has a focal length much longer than the focal length of lens **13** and the distance between the lenses; more particularly, $|f_{12}-z| \gg f_{13}$. In this embodiment, the displacement of the focal point is approximately

$$\delta \approx \frac{d \cdot f_{13}}{(f_{12} - z)}. \tag{6}$$

Given that $|f_{12}-z| >> f_{13}$, the displacement of the focal spot is greatly reduced relative to the displacement of the beam-adjusting lens **12**. This reduced sensitivity of the focal spot adjustment to movement of the beam-adjusting lens enables obtaining a high degree of precision over the adjustment of the focal spot using less expensive lens adjusting mechanisms with less precise motion control.

Preferably, the focal length of lens **12** is such that $|f_{12}-z| \geqq 2 \cdot f_{13}$, more preferably, $|f_{12}-z| \geqq 4 \cdot f_{13}$, and even more preferably, $|f_{12}-z| \geqq 6 \cdot f_{13}$. In general, the preferred focal length of lens **12** and distance z are selected based on the particular application, including the desired focal point adjustment sensitivity and the resolution of the lens adjusting mechanism.

Although FIGS. **1** and **2** depict the focusing optics as a single focusing lens (lens **13**), more complex optics may be used, such as, optics having multiple lens elements and, optionally, beam shaping optics, such as described in U.S. Pat. No. 4,498,766 and U.S. Patent Application Publication No. 2006-0256335, both incorporated herein by reference.

Dual-Lens Beam-Adjusting Optics

FIG. **3** shows a schematic representation of an embodiment of the illumination optics of the present invention in which the beam-adjusting optics consist of plano-concave lens **32** and plano-convex lens **33**, separated by a distance D. Light source **31** emits a beam having optical path **35**, which is focused to a focal spot **37** on the flow stream **36** by focusing lens **34**. One of lenses **32** and **33** is mounted such that the lens can be moved in a plane perpendicular to the optical path, such as by using a mechanical positioning system (not shown). In FIG. **3**, plano-concave lens **32** and plano-convex lens **33** are positioned such that the optical path is centered in each of the lenses, i.e., the optical axes of the lenses coincides with the optical path. In this configuration, the focal spot **37** of the illumination optics is not displaced by the beam-adjusting optics from the optical path **35**.

FIG. **4** shows a schematic representation of the beam illumination optics shown in FIG. **3**, wherein lens **33** has been displaced by a distance d from the optical path in a plane perpendicular to the optical path. The path of an arbitrary light ray **46** through the beam illumination optics is shown.

The optical effect of lens pair **32** and **33** on the illumination optics, relative to illumination optics having only focusing lens **34**, is two-fold. First, the addition of lens pair **32** and **33** modifies the focal length of the illumination optics. Second, displacement of one of the lenses of lens pair **32** and **33** in a plane perpendicular to the optical path displaces the focal point of the illumination optics.

From equation (1), above, the equivalent focal length, f_{eq}, of the lens pair **32** and **33** is

$$f_{eq} = \frac{f_{32} \cdot f_{33}}{f_{32} + f_{33} - D}, \tag{7}$$

wherein f_{32} and f_{33} are the focal lengths of lens **32** and lens **33**, respectively, and D is the distance between lens **32** and lens **33**.

From equation (3), above, the effective focal length, f_{eff}, of the illumination optics with lens pair **32** and **33** is

$$f_{eff} = \frac{f_{34}}{1 + \frac{f_{34}}{f_{eq} - z}}, \tag{8}$$

wherein f_{34} is the focal length of lens **34**, and z is the distance between second primary point of the lens pair **32** and **33** and lens **34**.

In a preferred embodiment, lenses **32** and **33** are matched, by which is meant that $f_{32}=-f_{33}$, and lenses **32** and **33** are separated by a small distance relative to the focal length of the lenses, i.e., $D << |f_{32}|$ and $D << |f_{33}|$. In this embodiment,

$$f_{eq} \approx \frac{-f_{32}^2}{D}, \tag{9}$$

and, thus, the equivalent focal length of the lens pair is much longer than the focal length of the individual lenses. Furthermore, equation (8) shows that, in this embodiment, the lens pair will have a negligible effect on the effective focal length of the illumination optics.

The displacement of the focal spot induced by a displacement of one of the lenses of the lens pair **32** and **33** can be obtained from an analysis of the optical property of the lens pair on the path of light ray **46** using the well-known ray tracing technique of ray transfer matrix analysis (see, for example, Warren J. Smith, 1996, Modern Optical Engineering: The Design of Optical Systems, 2nd Ed. (McGraw-Hill, Inc., New York, N.Y.), incorporated herein by reference). In ray transfer matrix analysis (also known as ABCD matrix analysis), an optical system (e.g., one or more lenses) is described using a ray transfer matrix, and a vector representing the light ray leaving the system is determined by multiplying the ray transfer matrix with a vector representing the light ray entering the system. The technique uses the paraxial approximation of ray optics in which a ray is assumed to be at a small angle (θ) to the optical axis of the system and remain at a small distance (x) from the optical axis of the system. This allows the approximations $\sin(\theta) \approx \theta$, $\tan(\theta) \approx \theta$, and $\cos(\theta) \approx 1$ (where θ is measured in radians) to be used in the calculation of the ray's path. A thin-lens approximation (see above) is also used in the following analysis.

In ray transfer matrix analysis, an arbitrary paraxial light ray is specified by the vector

$$\begin{pmatrix} x \\ \theta \end{pmatrix},$$

wherein x is the distance of the ray from the optical axis, and θ is the angle between the ray and the optical axis. The ray vector after passing through an optical system, denoted by

$$\begin{pmatrix} x' \\ \theta' \end{pmatrix},$$

is then

$$\begin{pmatrix} x' \\ \theta' \end{pmatrix} = S \begin{pmatrix} x \\ \theta \end{pmatrix},$$

wherein S is the ray transfer matrix for the optical system.

For a paraxial ray impinging upon a thin lens of focal length f that is displaced a distance d from the optical axis of the beam,

$$\begin{pmatrix} x' \\ \theta' \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ -1/f & 1 \end{pmatrix} \begin{pmatrix} x \\ \theta \end{pmatrix} + \begin{pmatrix} 0 \\ -d/f \end{pmatrix}.$$

Denote the focal lengths of lenses **32** and **33** as f_{32} and f_{33}, respectively. Then, for a paraxial ray impinging on lens pair **32** and **33**, separated by a distance D, where lens **33** is displaced d from the optical axis,

$$\begin{pmatrix} x' \\ \theta' \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ -1/f_{33} & 1 \end{pmatrix} \begin{pmatrix} 1 & D \\ 0 & 1 \end{pmatrix} \begin{pmatrix} 1 & 0 \\ -1/f_{32} & 1 \end{pmatrix} \begin{pmatrix} x \\ \theta \end{pmatrix} + \begin{pmatrix} 0 \\ d/f_{33} \end{pmatrix}.$$

In a preferred embodiment, lenses **32** and **33** are matched, by which is meant that $f_{32}=-f_{33}$, and lenses **32** and **33** are separated by a small distance relative to the focal length of the lenses, i.e., $D<<|f_{32}|$ and $D<<|f_{33}|$. In this embodiment, any ray that is nearly parallel to the optical axis (i.e., for which $\theta\approx\theta$) will then be transformed by the lens pair into

$$x' \approx (1+D/f_{33}) \cdot x \tag{10}$$

$$\theta' \approx d/f_{33}$$

The angular displacement induced by the lens pair, given in equation (10), above, causes a displacement, δ, of the focal spot of the illumination optics, where

$$\delta = f_{34} \cdot \theta' \approx \frac{f_{34} \cdot d}{f_{33}}. \tag{11}$$

Using long focal length lenses **32** and **33**, such that that $f_{34}/f_{33}<<1$, the displacement of the focal spot is greatly reduced relative to the displacement of the beam-adjusting lens **33**. This reduced sensitivity of the focal spot adjustment to movement of the beam-adjusting lens enables obtaining a high degree of precision over the adjustment of the focal spot using less expensive lens adjusting mechanisms with less precise motion control.

I claim:

1. An optical analyzer comprising:

(a) a light source adapted to emit an approximately collimated light beam along a light path;

(b) a focusing lens positioned in the light path, adapted to focus the light beam onto a focal spot within a sample analysis region, wherein said focusing lens has a focal length f_1,

(c) beam-adjusting optics positioned in the light path between the light source and the focusing lens, wherein said beam-adjusting optics comprises at least one beam-adjusting lens that is mounted in a positioning device that allows movement of the beam-adjusting lens in a plane perpendicular to the light path, wherein said beam-adjusting lens has a focal length f_2, wherein said beam-adjusting lens and said focusing lens are separated by a distance z along the light path, and wherein $|f_2-z|\geqq 4\cdot f_1$.

2. The optical analyzer of claim **1**, wherein $|f_2-z|\geqq 6\cdot f_1$.

3. The optical analyzer of claim **1**, wherein said beam-adjusting lens is a spherical lens.

4. The optical analyzer of claim **1**, wherein said beam-adjusting lens is a cylindrical lens.

5. An optical analyzer comprising:

(a) a light source adapted to emit an approximately collimated light beam along a light path;

(b) a focusing lens positioned in the light path, adapted to focus the light beam onto a focal spot within a sample analysis region, wherein said focusing lens has a focal length f_1,

(c) beam-adjusting optics positioned in the light path between the light source and the focusing lens, wherein said beam-adjusting optics comprises at least one beam-adjusting lens that is mounted in a positioning device that allows movement of the beam-adjusting lens in a plane perpendicular to the light path, wherein said beam-adjusting optics comprise a divergent lens having a focal length f_2, wherein f_2 is negative, and a convergent lens having a focal length f_3, wherein f_3 is positive, wherein said beam-adjusting lens is said divergent lens or said convergent lens, wherein $f_2=-f_3$.

6. An optical analyzer comprising:

(a) a light source adapted to emit an approximately collimated light beam along a light path;

(b) a focusing lens positioned in the light path, adapted to focus the light beam onto a focal spot within a sample analysis region, wherein said focusing lens has a focal length f_1,

(c) beam-adjusting optics positioned in the light path between the light source and the focusing lens, wherein said beam-adjusting optics comprises at least one beam-adjusting lens that is mounted in a positioning device that allows movement of the beam-adjusting lens in a plane perpendicular to the light path, wherein said beam-adjusting optics comprise a plano-concave lens having a focal length f_2 and a plano-convex lens having a focal length f_3, wherein said beam-adjusting lens is said plano-concave lens of said plano-convex lens.

7. The optical analyzer of claim **6**, wherein $f_2\geqq 2\cdot f_1$ and $-f_3\geqq 2\cdot f_1$.

8. The optical analyzer of claim **6**, wherein $f_2\geqq 4\cdot f_1$ and $-f_3\geqq 4\cdot f_1$.

9. The optical analyzer of claim **6**, wherein $f_2\geqq 6\cdot f_1$ and $-f_3\geqq 6\cdot f_1$.

10. The optical analyzer of claim **6**, wherein $f_2=-f_3$.

11. The optical analyzer of claim **10**, wherein $f_2\geqq 2\cdot f_1$.

12. The optical analyzer of claim **10**, wherein $f_2\geqq 4\cdot f_1$.

13. The optical analyzer of claim **10**, wherein $f_2\geqq 6\cdot f_1$.

* * * * *

EXHIBIT 5

CytoFLEX Flow Cytometer

The CytoFLEX Flow Cytometer, the first introduction to the CytoFLEX Platform, provides the performance you need in an easy to use system allowing you to focus on the science, not the instrumentation. Its superior sensitivity and resolution throughout all configurations give it the edge over other cytometry systems four times its size. All instruments in the platform utilize CytExpert for CytoFLEX Acquisition and Analysis Software.

- Violet-Blue-Red (V-B-R) Series, fully activated with 13 colors or as few as four, activate additional channels in the future
- Includes 13 band pass filters: 450/45, 525/40 (2), 585/42, 610/20 (2), 660/10 (2), 690/50, 712/25, 780/60 (3)
- For higher throughput applications an optional plate loader module is available and for use in regulated environments the software contains tools for 21 CFR Part 11 compliance

CytoFLEX Flow Cytometer Features

Exquisite Sensitivity

- Unique collection of innovative technologies provide exceptional detection capabilities
- Large dynamic range to resolve dim and bright populations in the same sample
- Use violet side scatter to detect nanoparticles

Platform Versatility

- Purchase the number of parameters needed now
- Add more detection channels by purchasing an activation key
- Includes full complement of repositionable bandpass filters, non-standard filters also available for even more flexibility

Low Maintenance

- Lasers, steering optics and flow cell are all mounted onto a vibration isolated table, maintaining optical alignment
- Low pressure fluidics system
- Preventive Maintenance can be completed by the user, no service visit required

Automation Enabled

- Optional 96-well plate sample loader module
- API (Application Programming Interface) allows external software to control the instrument
- For automated sample processing and data acquisition integrate with the Biomek i-Series Instrument

Choose a CytoFLEX Model

Intended Use

RUO	25

Part Number

C09738	1
C09748	1
C09747	1
C09746	1
C09741	1

Search

Product Line Regulatory

- [ ] CytoFlex 25

Category

- [ ] CytoFLEX 25

Regulatory Status

- [ ] RUO 25

Optics and Laser Power

- [ ] 488 nm solid state laser (50 mW) 24
- [ ] 638 nm solid state laser (50 mW) 20
- [ ] 405 nm solid state laser (80 mW) 17
- [ ] 488 nm solid state laser (25 mW) 1

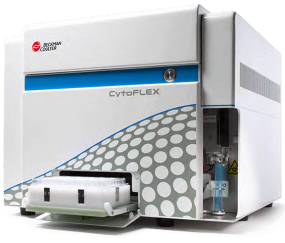
Package Quantity

- [ ] 1 25

Power Requirements

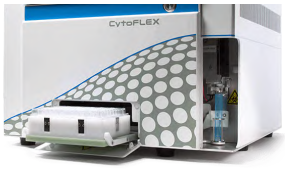
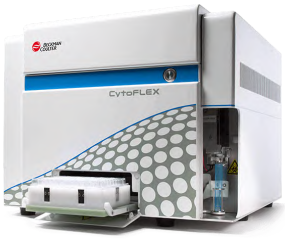
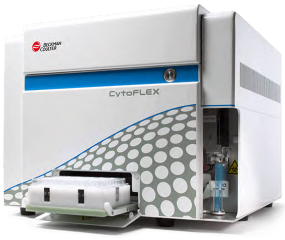
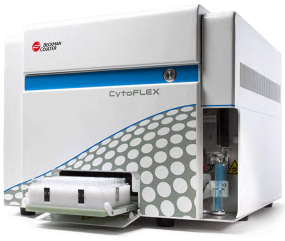
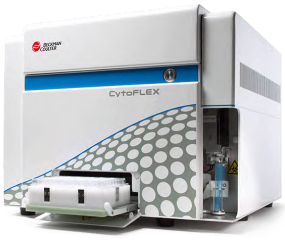
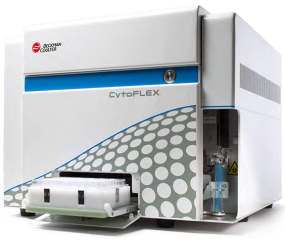
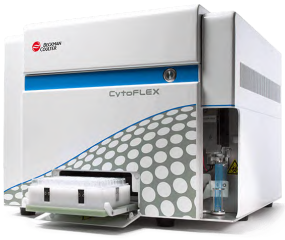
- [ ] 50/60 Hz 25
- [ ] 100-240 VAC 25

Results **1-8** of **25**



CytoFLEX V0-B4-R2 Flow Cytometer (6 Detectors, 2 Lasers)

CytoFLEX V3-B3-R0 Flow

	Cytometer (6 Detectors, 2 Lasers)
	CytoFLEX V0-B3-R3 Flow Cytometer (6 Detectors, 2 Lasers)
	CytoFLEX V3-B4-R3 Flow Cytometer (10 Detectors, 3 Lasers)
	CytoFLEX V2-B3-R3 Flow Cytometer (8 Detectors, 3 Lasers)
	CytoFLEX V2-B4-R3 Flow Cytometer (9 Detectors, 3 Lasers)
	CytoFLEX V3-B3-R3 Flow Cytometer (9 Detectors, 3 Lasers)
	CytoFLEX V2-B3-R2 Flow Cytometer (7 Detectors, 3 Lasers)

CytoFLEX Flow Cytometer Specifications

Detection	Photodiodes
Resolution	<300 nm particles (488 nm side scatter)
Performance	<30 MESF for FITC, <10 MESF for PE
Flow Cell	Patent-pending, alignment-free integrated optics quartz flow cell (420 µm x 180 µm ID) design with >1.3 numerical aperture
Signal Processing	Digital system with 7-decade display
Power Requirements	100-240 VAC, 50/60 Hz
Item Specifications Referenced	C09751

Related Parts

B53230, CytoFLEX Daily QC Fluorospheres

$318.00

C06147, CytoFLEX Daily IR QC Fluorospheres

$369.00

B51503, CytoFLEX Sheath Fluid, 1 x 10 L

$29.00

Life Sciences Division Headquarters
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United States

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PRODUCT AVAILABILITY AND REGULATORY STATUS DEPENDS ON COUNTRY REGISTRATION PER APPLICABLE REGULATIONS

The listed regulatory status for products correspond to one of the below:
IVD: In Vitro Diagnostic Products. These products are labeled "For In Vitro Diagnostic Use."
ASR: Analyte Specific Reagents. These reagents are labeled "Analyte Specific Reagents. Analytical and performance characteristics are not established."
CE: Products intended for in vitro diagnostic use and conforming to European Directive (98/79/EC). (Note: Devices may be CE marked to other directives than (98/79/EC)
RUO: Research Use Only. These products are labeled "For Research Use Only. Not for use in diagnostic procedures."
LUO: Laboratory Use Only. These products are labeled "For Laboratory Use Only."
No Regulatory Status: Non-Medical Device or non-regulated articles. Not for use in diagnostic or therapeutic procedures.

EXHIBIT 6

CytoFLEX S Flow Cytometer

The CytoFLEX S Flow Cytometer series is an expansion of the CytoFLEX Platform. This model expands research possibilities, with a fourth laser option, multiple configurations and unique filter sets that allows investigators to focus on the science, not the instrumentation. Four different series are offered in this model. All instruments in the platform utilize CytExpert for CytoFLEX Acquisition and Analysis Software.

- The four series are Violet-Blue-Yellow Green-Red (V-B-Y-R), Near UV-Violet-Blue-Red (N-V-B-R),Near UV-Violet-Blue-Yellow Green (N-V-B-Y), Violet-Blue-Red-IR (V-B-R-I)
- Activate as few as four parameters and upgrade later within the series up to 13 parameters
- For higher throughput applications an optional plate loader module is available and for use in regulated environments the software contains tools for 21 CFR Part 11 compliance

CytoFLEX S Flow Cytometer Features

Exquisite Sensitivity

- Unique collection of innovative technologies provide exceptional detection capabilities
- Large dynamic range to resolve dim and bright populations in the same sample
- Use violet side scatter to detect nanoparticles

Platform Versatility

- Purchase the number of parameters needed now
- Add more parameters by purchasing an activation key
- Includes full complement of repositionable bandpass filters, non-standard filters also available for even more flexibility

Low Maintenance

- Lasers, steering optics and flow cell are all mounted onto a vibration isolated table, maintaining optical alignment
- Low pressure fluidics system
- Preventive Maintenance can be completed by the user, no service visit required

Automation Enabled

- Optional 96-well plate sample loader module
- API (Application Programming Interface) allows external software to control the instrument
- For automated sample processing and data acquisition integrate with the Biomek i-Series Instrument

Choose a CytoFLEX S Model

Intended Use

RUO	19

Part Number

C01160	1
B78561	1
B78560	1
B78559	1
B78558	1

Search

Product Line Regulatory

- [ ] CytoFlex 19

Category

- [ ] CytoFLEX S 19

Regulatory Status

- [ ] RUO 19

Optics and Laser Power

- [ ] 488 nm solid state laser (50 mW) 19
- [ ] 561 nm solid state laser (30 mW) 11
- [ ] 405 nm solid state laser (80 mW) 9
- [ ] 638 nm solid state laser (50 mW) 9
- [ ] 375 nm solid state laser (60 mW) 5
- [ ] Search

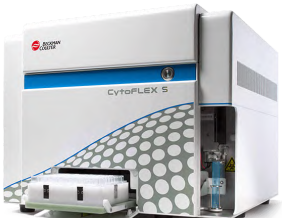
Package Quantity

- [ ] 1 19

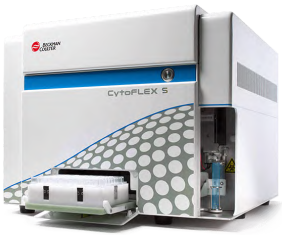
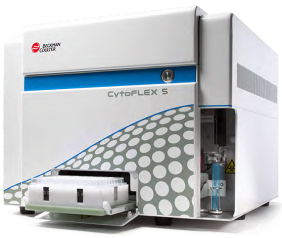
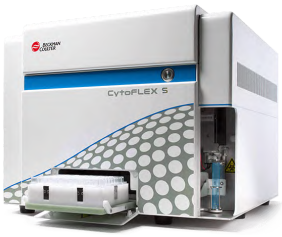
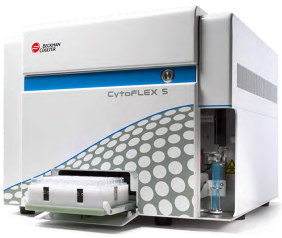
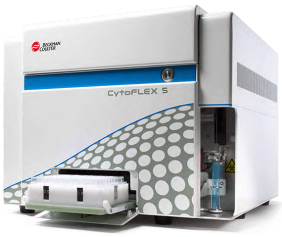
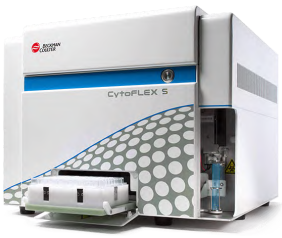
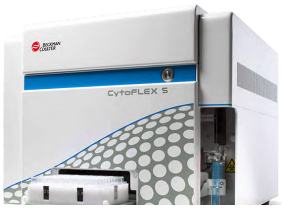
Power Requirements

- [ ] 50/60 Hz 19
- [ ] 100-240 VAC 19

Results **1**-**8** of **19**



CytoFLEX S V0-B4-R3-I2 Flow Cytometer (9 Detectors, 3 Lasers)

	CytoFLEX S V0-B4-R0-I2 Flow Cytometer (6 Detectors, 2 Lasers)
	CytoFLEX S V4-B4-R0-I2 Flow Cytometer (10 Detectors, 3 Lasers)
	CytoFLEX S V4-B4-R3-I2 Flow Cytometer (13 Detectors, 4 Lasers)
	CytoFLEX S V0-B2-Y4-R3 Flow Cytometer (9 Detectors, 3 Lasers)
	CytoFLEX S V4-B2-Y4-R0 Flow Cytometer (10 Detectors, 3 Lasers)
	CytoFLEX S V0-B2-Y4-R0 Flow Cytometer (6 Detectors, 2 Lasers)
	CytoFLEX S V4-B2-Y0-R3 Flow Cytometer (9 Detectors, 3 Lasers)

CytoFLEX S Flow Cytometer Specifications

Detection	Photodiodes
Resolution	<300 nm particles (488 nm side scatter)
Performance	<30 MESF for FITC
Flow Cell	Patent-pending, alignment-free integrated optics quartz flow cell (420 µm x 180 µm ID) design with >1.3 numerical aperture
Signal Processing	Digital system with 7-decade display
Power Requirements	100-240 VAC, 50/60 Hz
Item Specifications Referenced	C02949

Related Parts

B53230, CytoFLEX Daily QC Fluorospheres

$318.00

C06147, CytoFLEX Daily IR QC Fluorospheres

$369.00

B51503, CytoFLEX Sheath Fluid, 1 x 10 L

$29.00

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IVD: In Vitro Diagnostic Products. These products are labeled "For In Vitro Diagnostic Use."
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CE: Products intended for in vitro diagnostic use and conforming to European Directive (98/79/EC). (Note: Devices may be CE marked to other directives than (98/79/EC)
RUO: Research Use Only. These products are labeled "For Research Use Only. Not for use in diagnostic procedures."
LUO: Laboratory Use Only. These products are labeled "For Laboratory Use Only."
No Regulatory Status: Non-Medical Device or non-regulated articles. Not for use in diagnostic or therapeutic procedures.

EXHIBIT 7

CytoFLEX LX Flow Cytometer

The CytoFLEX LX Flow Cytometer is an expansion of the CytoFLEX Platform. This model expands research possibilities with up to six lasers and 21 color parameters. Six spatially separated lasers allows panels to be spread across the spectrum reducing cross talk and spectral overlap. All instruments in the platform utilize CytExpert for CytoFLEX Acquisition and Analysis Software.

- Two series are available, UV-Violet-blue-Yellow Green-Red-IR (U-V-B-Y-R-I) and Near UV-Violet-Blue-Yellow Green-Red-IR (N-V-B-Y-R-I)
- Instruments with as few as four activated laser and 14 color parameters are available with the option to activate additional lasers and detection channels in the future with the purchase of an activation key
- For higher throughput applications an optional plate loader module is available and for use in regulated environments the software contains tools for 21 CFR Part 11 compliance

CytoFLEX LX Flow Cytometer Features

Exquisite Sensitivity

- Unique collection of innovative technologies provide exceptional detection capabilities
- Large dynamic range to resolve dim and bright populations in the same sample

- Use violet side scatter to detect nanoparticles

Platform Versatility

- Purchase the number of parameters needed now and add more parameters by purchasing an activation key
- Includes full complement of repositionable bandpass filters, non-standard filters also available for even more flexibility
- CytoFLEX LX WDM Beam Splitter module available to assign IR channels to UV, Near UV, or Violet laser WDM

Low Maintenance

- Lasers, steering optics and flow cell are all mounted onto a vibration isolated table, maintaining optical alignment
- Low pressure fluidics system
- Preventive Maintenance can be completed by the user, no service visit required

Automation Enabled

- Optional 96-well plate sample loader
- API (Application Programming Interface) allows external software to control the instrument
- For automated sample processing and data acquisition integrate with the Biomek i-Series Instrument

Choose a CytoFLEX LX Model

Intended Use

RUO	7

Part Number

C40312	1
C40315	1
C40313	1
C40323	1

C40322

- [ ] Search 1

Product Line Regulatory

- [ ] CytoFlex 7

Category

- [ ] CytoFLEX LX 7

Regulatory Status

- [ ] RUO 7

Optics and Laser Power

- [ ] 405 nm solid state laser (80 mW) 7
- [ ] 488 nm solid state laser (50 mW) 7
- [ ] 561 nm solid state laser (30 mW) 6
- [ ] 638 nm solid state laser (50 mW) 6
- [ ] 355 nm solid state laser (20 mW) 4
- [ ] Search

Package Quantity

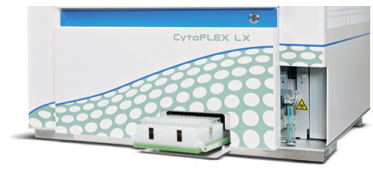
- [ ] 1 7

Power Requirements

- [ ] 50/60 Hz 7
- [ ] 100-240 VAC 7

Results **1-7** of **7**

CytoFLEX LX

	U3-V5-B3-Y0-R3-I0 Flow Cytometer (14 Detectors, 4 Lasers)
	CytoFLEX LX U3-V5-B3-Y5-R3-I2 Flow Cytometer (21 Detectors, 6 Lasers)
	CytoFLEX LX U3-V5-B3-Y5-R0-I0 Flow Cytometer (14 Detectors, 4 Lasers)
	CytoFLEX LX U3-V5-B3-Y5-R3-I0 Flow Cytometer (19 Detectors, 5 Lasers)
	CytoFLEX LX N3-V5-B3-Y5-R3-I0 Flow Cytometer (19 Detectors, 5 Lasers)
	CytoFLEX LX N0-V5-B3-Y5-R3-I0 Flow Cytometer (16 Detectors, 4 Lasers)
	CytoFLEX LX N3-V5-B3-Y5-R3-I2 Flow Cytometer (21 Detectors, 6 Lasers)

CytoFLEX LX Flow Cytometer Specifications

Detection	Photodiodes
Resolution	<300 nm particles (488 nm side scatter), <80 nm polystyrene particles (405 nm side scatter), rCV 3%
Performance	<30 MESF for FITC, <10 MESF for PE
Flow Cell	Patent-pending, alignment-free integrated optics quartz flow cell (420 µm x 180 µm ID) design with >1.3 numerical aperture
Signal Processing	Digital system with 7-decade display
Power Requirements	100-240 VAC, 50/60 Hz
Item Specifications Referenced	C40312

Related Parts

B53230, CytoFLEX Daily QC Fluorospheres

$318.00

C06147, CytoFLEX Daily IR QC Fluorospheres

$369.00

B51503, CytoFLEX Sheath Fluid, 1 x 10 L

$29.00

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EXHIBIT 8

CytoFLEX SRT Benchtop Cell Sorter

CytoFLEX SRT Cell Sorter is a benchtop sorter. It is capable of meeting requirements for a wide range of sorting needs. And like the CytoFLEX Platform, it includes innovative technologies that simplify the setup and operation, empowering investigators to focus on the research questions. The Violet-Blue-Yellow Green-Red (V-B-Y-R) Series has 15 fluorescent detectors when fully activated. It can be purchased with as few as four with an option to activate additional lasers and detectors with an activation key. It is capable of complex sort logic with different combinations of sort settings on each of four streams, including the ability to catch aborts of one of the other streams. An optional Biosafety Cabinet including aerosol evacuation or a standalone Aerosol Evacuation Unit is available.

- Capable of complex sort logic, including 4-way sorting, Mixed Mode sorting, and the ability to catch aborts and preserve precious cells
- Setup is smart and simplified, using automation to establish and maintain the sort stream
- Remote support ready using BeckmanConnect for on demand access to our technical specialists, perform many routine maintenance procedures independently

CytoFLEX SRT Features

CytoFLEX that Sorts

- Exquisite sensitivity for multicolor applications
- Parameter matched to the CytoFLEX S V-B-Y-R Series
- Intuitive software to facilitate multicolor analysis, CytExpert for CytoFLEX SRT

Accessible Sorting

- From startup to sample sorting in <30 minutes
- Uses technology to increase ease of use by automating sort stream setup and maintenance throughout the sort
- Designed for reliability and supported by teams of experts

Complex Sort Logic

- Up to 15 fluorescent colors to define populations
- 4-way sorting, Mixed Mode sorting
- Ability to catch aborts and preserve precious cells

Delivering Quality Cells

- High recovery and post sort viability
- Accurate single-cell deposition using the Cyclone Movement System, including Index Sorting
- Sort into tubes, slides, or microplates

Choose a CytoFLEX SRT Model

Part Number	
C71889	1
C71887	1
C71886	1
C71884	1
C71883	1

Product Line Regulatory	
CytoFLEX Sorter	5

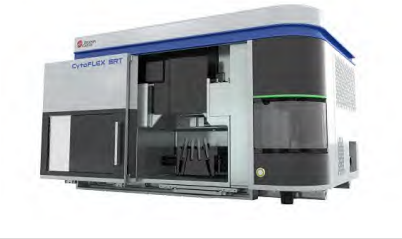
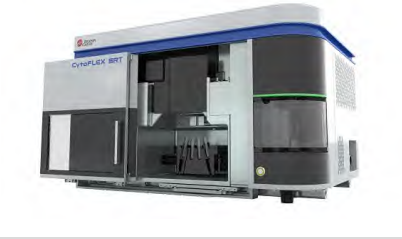
Regulatory Status

- [ ] RUO **5**

Power Requirements

- [ ] 100-240 VAC, 50/60 Hz **5**

Results **1-5** of **5**

	CytoFLEX SRT V5-B2-Y5-R3 Cell Sorter (15 Colors, 4 Lasers)
	CytoFLEX SRT V5-B2-Y5-R0 Cell Sorter (12 Colors, 3 Lasers)
	CytoFLEX SRT V0-B2-Y5-R3 Cell Sorter (10 Colors, 3 Lasers)
	CytoFLEX SRT V5-B2-Y0-R0 Cell Sorter (7 Colors, 2 Lasers)
	CytoFLEX SRT V0-B2-Y0-R3 Cell Sorter (5 Colors, 2 Lasers)

CytoFLEX SRT Benchtop Cell Sorter Specifications

Signal Processing	Digital system with 7-decade display
Resolution	300 nm particles (488 nm SSC), 200 nm particles (405 nm VSSC), rCV 3%
Performance	<30 MESF for FITC, <10 MESF for PE, <25 MESF for APC
Detection	Avalanche Photodiodes
Lasers	488 nm, 561 nm, 638 nm
Optical Filters	450/45, 525/40 (2), 585/42, 610/20 (2), 660/10 (2), 675/30, 690/50, 710/50, 712/25, 780/60 (3)
Sorting Performance	With a target population of 5% and a threshold rate of <10,000 events per second, >99% purity and >80% of Poisson's expected yield
Nozzle Diameter(s)	100 µm
Drop Dive Frequency	Automatically set, manually adjustable between 30,000-35,000 Hz
Collection Vessels	12x75 mm tubes, 15 mL conical tubes, 96-well plates, 96-deep-well plates, 384-well plates, slides
Sorting Modes	4-way Sorting; enrichment or purity or single, ability to catch aborts
Power Requirements	100-240 VAC, 50/60 Hz
Item Specifications Referenced	C71886

Related Products or Parts

Life Sciences Division Headquarters
5350 Lakeview Parkway S Drive
Indianapolis, IN 46268
United States

NOT ALL PRODUCTS ARE AVAILABLE IN ALL COUNTRIES.
PRODUCT AVAILABILITY AND REGULATORY STATUS DEPENDS ON COUNTRY REGISTRATION PER APPLICABLE REGULATIONS

The listed regulatory status for products correspond to one of the below:
IVD: In Vitro Diagnostic Products. These products are labeled "For In Vitro Diagnostic Use."
ASR: Analyte Specific Reagents. These reagents are labeled "Analyte Specific Reagents. Analytical and performance characteristics are not established."
CE: Products intended for in vitro diagnostic use and conforming to European Directive (98/79/EC). (Note: Devices may be CE marked to other

directives than (98/79/EC)
RUO: Research Use Only. These products are labeled "For Research Use Only. Not for use in diagnostic procedures."
LUO: Laboratory Use Only. These products are labeled "For Laboratory Use Only."
No Regulatory Status: Non-Medical Device or non-regulated articles. Not for use in diagnostic or therapeutic procedures.

EXHIBIT 9

Consumables for the CytoFLEX Platform

Startup kits are available to ensure that when your unit arrives you will be ready to start your experiments. We also offer preventive maintenance kits and consumables for routine use and maintenance.

Description	Part #
Startup Kits	
CytoFLEX Startup Reagents - (Daily QC Beads, Sheath, Flow Clean, Conrad, Tubes)	B55031
CytoFLEX Startup Reagents - PL (Daily QC Beads, Sheath, Flow Clean, Contrad, Plates)	C14907

CytoFLEX Startup Reagents - Infrared Systems (Daily QC Beads, IR QC beads, Sheath, Flow Clean, Conrad, Tubes)	C14908
CytoFLEX Startup Reagents - Infrared Systems PL (Daily QC Beads, IR QC beads, Sheath, Flow Clean, Contrad, Plates)	C14909

Consumables

CytoFLEX Daily QC Fluorospheres Download QC target files	B53230
CytoFLEX Daily IR QC Fluorospheres Download QC target files	C06147
CytoFLEX Sheath Fluid	B51503
FlowClean Cleaning Agent	A64669
Contrad 70	81911
Polypropylene Sample Tubes, Blue	2523749
Microtiter Plates, 96-well Round Deep Well Plates	267001
Microtiter Plates, 96-well Round Deep Well Plates	267006
Microtiter Plates, 96-well Square Deep Well Plates	140504
Microtiter Plates, 96-well Flat Bottom	609844
Microtiter Plates, 96-well V Bottom	609801

Preventive Maintenance Kits

Preventive Maintenance Kit	C02943
Peristaltic Sample Tubing Replacement Kit (every 6 months)	A04-1-0048

Sheath Filter (for Preventive Maintenance)	A04-1-0041

Replacement Parts

Plate Loader Sample Probe (with tubing to attach to plate assembly)	B63213
Sample Needle, 113 mm (orange bead)	B71294
Sample Needle, 115 mm (blue bead)	A04-1-0034
Deep Clean Solution Bottle Kits	A04-1-0038
Sheath Bottle Kit	A04-1-0036
Waste Bottle Kit	A04-1-0037

*** Product Interest**

Please select...

*** How Can We Help**

Please select...

*** First Name**

*** Last Name**

*** Email**

Phone

* Company

* Country

* Postal Code

* Address

* City

* Comments

Please keep me informed about Beckman Coulter webinars, products, goods, and services, including products, goods, or services from our related companies.

* By Email: ◯ **I Consent**
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INSTRUMENTS +

REAGENTS +

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No Regulatory Status: Non-Medical Device or non-regulated articles. Not for use in diagnostic or therapeutic procedures.

EXHIBIT 10

CytoFLEX SRT Cell Sorter Consumables

Startup Packs	*Part #*
CytoFLEX SRT Startup Reagent Bundle Kit - A Startup Pack is available to ensure that when your unit arrives you will be ready to start your experiments. We also offer consumables for routine use and maintenance.	C71882

Reagents	*Part #*
Contrad 70 Cleaning Solution	81911
CytoFLEX Daily QC Fluorospheres	B53230

CytoFLEX Sheath Fluid	B51503
FlowClean Cleaning Agent	A64669
FP,FLOW CHECK KIT COULTER FLUOROSPHERES 3 X 10 ML KIT	6605359
IsoFlow Sheath Fluid	8546859
CytoFLEX SRT Shutdown Fluid	C52574

Plastics	*Part #*
Polypropylene Sample Tubes, Blue	2523749
Microtiter Plates, 96-well V Bottom	609801
Microtiter Plates, 96-well Flat Bottom	609844

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ASR: Analyte Specific Reagents. These reagents are labeled "Analyte Specific Reagents. Analytical and performance characteristics are not established."
CE: Products intended for in vitro diagnostic use and conforming to European Directive (98/79/EC). (Note: Devices may be CE marked to other directives than (98/79/EC)
RUO: Research Use Only. These products are labeled "For Research Use Only. Not for use in diagnostic procedures."
LUO: Laboratory Use Only. These products are labeled "For Laboratory Use Only."
No Regulatory Status: Non-Medical Device or non-regulated articles. Not for use in diagnostic or therapeutic procedures.

EXHIBIT 11

CytoFLEX Flow Cytometer Platform

Join the Resolution REVOLUTION

EVERY
event matters.

BECKMAN COULTER
Life Sciences

Benchtop Cytometry without Compromises

The CytoFLEX Platform is a revolutionary system presenting optimal excitation and emission, minimizing light loss and maximizing sensitivity. Since its initial unveiling, the compact system with innovative technology borrowed from the telecommunications industry has garnered attention from the flow cytometry community. Since that time, we have continued to expand the platform, creating even more choices for researchers.

We continue to leverage the power of the platform:

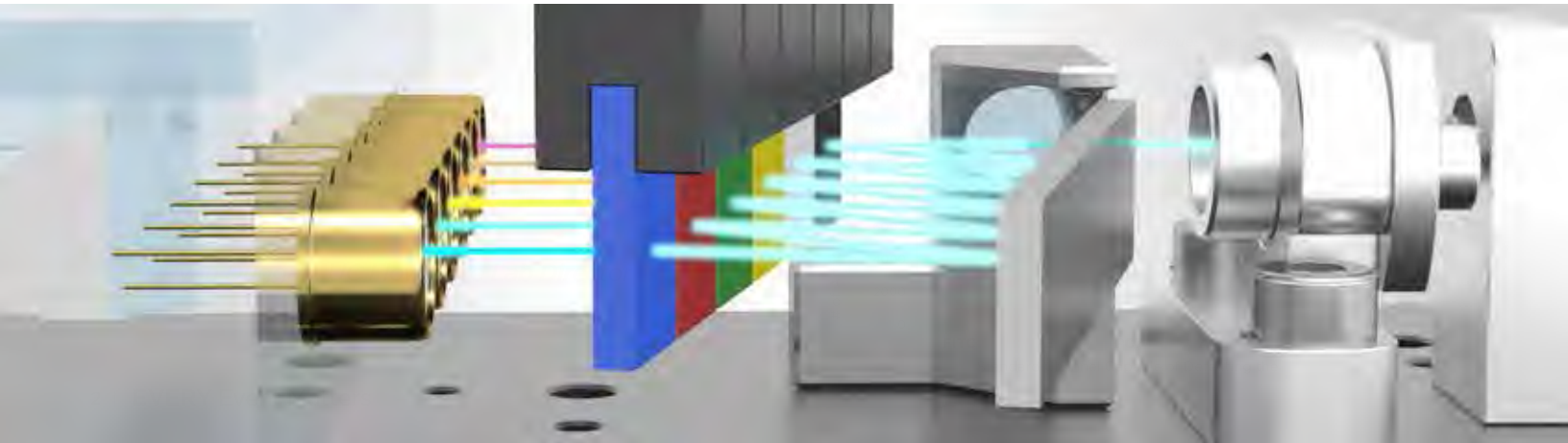
- Exquisite sensitivity
- Small particle analysis in a benchtop analyzer
- Extensive set of repositionable band pass filters
- Flexibility to upgrade by adding additional parameters
- Intuitive software to facilitate multicolor analysis

Up to 3 lasers

Up to 4 lasers

Up to 6 lasers

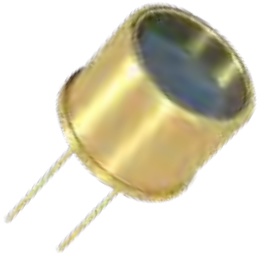
Visit Beckman.com/cytoflex

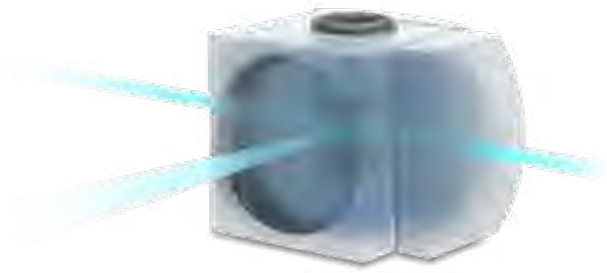


Harness the Power of Advanced Sensitivity

A unique assembly of technologies contributes to the exquisite sensitivity of the platform. Borrowing technology from the telecommunications industry, the Wavelength Division Multiplexer (WDM) deconstructs and measures multiple wavelengths of light. The WDM relies on fiber optics and band pass filters to separate the light wavelengths. Unlike more traditional instruments, multiple dichroic filters to direct the light path are not required. This makes it much easier to configure the fluorescence channels, but also increases light efficiency as light loss due to refraction is minimized.

The WDM utilizes Avalanche Photodiode detectors (APD), versus Photomultiplier tubes (PMT). One hallmark of the photodiode is the high quantum efficiency in excess of 80%, especially for wavelengths greater than 800 nm.

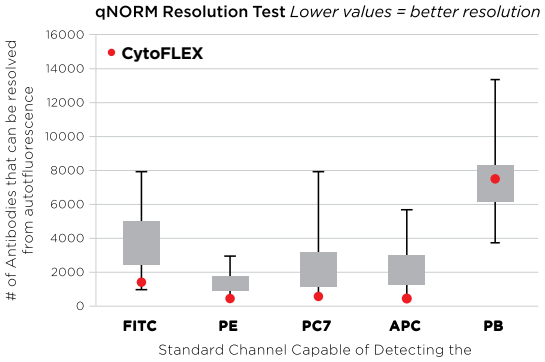




With conventional analyzers, laser excitation sources are optimized by shaping and focusing light through a series of lenses and filters onto the flow cell. Each of these light interactions is an opportunity for light loss. Another component of the system which increases efficiency is the use of integrated optics to focus light onto the flow cell. All of these technologies work together in the CytoFLEX to ensure efficient light management for optimal excitation and emission of fluorochrome-tagged cells, which is critical to its performance.

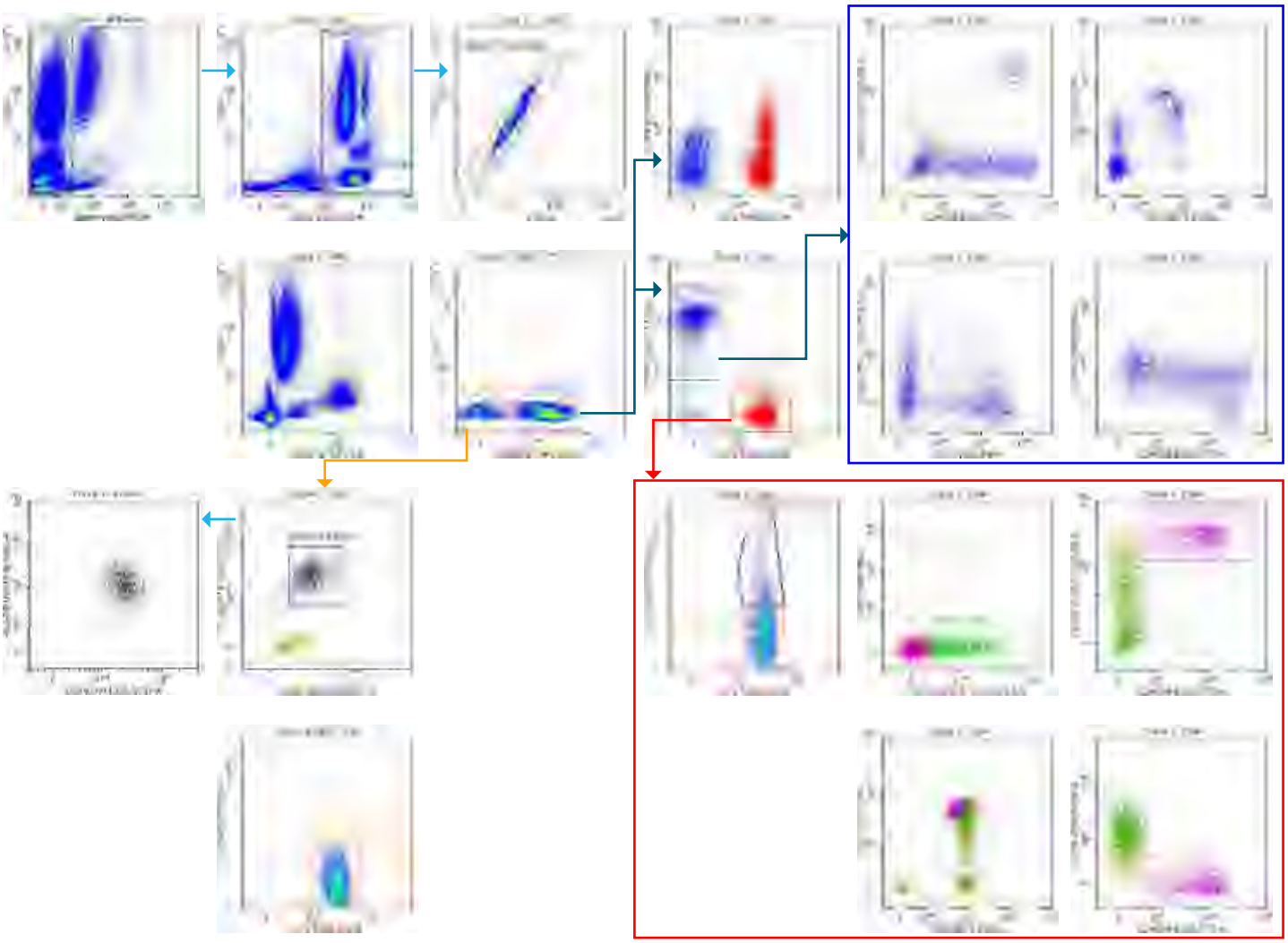
"The CytoFLEX compares very well with all the best instruments out there. It definitely beats every instrument I own in the FITC, PE, PECy7, and APC channels."

Ryan Duggan, UC Flow Core Lab Director



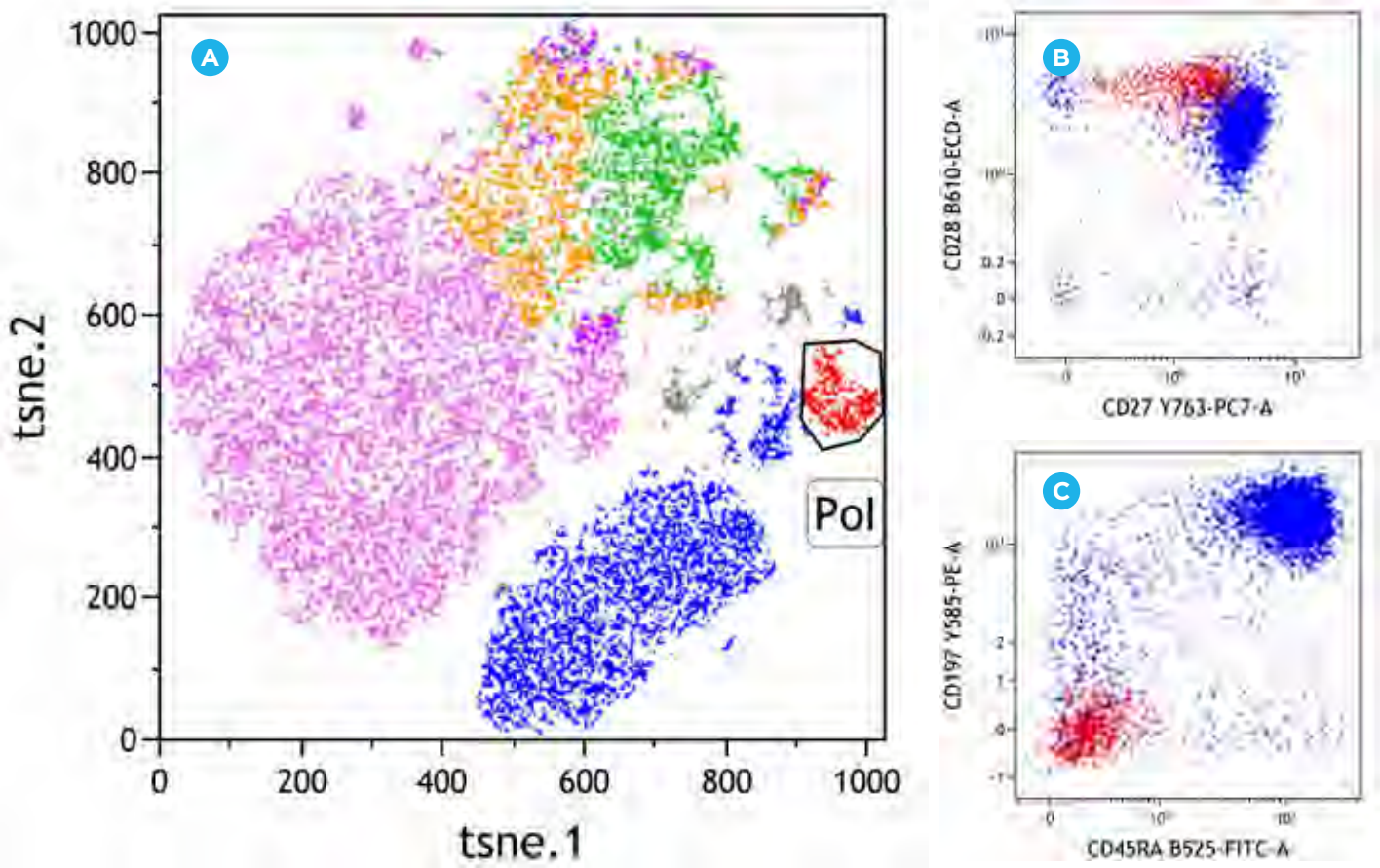
Focus on the Science

The CytoFLEX Platform innovative design delivers powerful performance in a compact, easy-to-use flow cytometer. It simplifies the practice of flow cytometry so that it can be more readily used by a wider range of scientists, allowing them to harness the power of single cell analysis. Increasing the robustness of the system, detectors, light management, fluidics and compensation algorithms means that establishing multicolor assays takes less planning and optimization.



375 NM			405 NM					488 NM			561 NM					638 NM			808 NM	
405/ 305	675/ 30	740/ 35	450/ 45	525/ 40	601/ 20	660/ 10	763/ 43	525/ 40	610/ 20	690/ 50	585/ 42	610/ 20	675/ 20	710/ 50	763/ 43	660/ 10	712/ 25	763/ 43	840/ 20	885/ 40
BUV395	BUV661	BUV737	PAC BLUE	KROME ORANGE	BV605	BV650	BV786	FITC	ECD	B690	PE	Y610	PC5	PC5.5	PC7	APC	APC-A700	APC-A750	IR840	IR885/ 40
CD20	HLA-DR	CD19	CD57	CD45	CCR4	CD95	CD25	CD45RA	CD28		CCR7		CD33	CD279 (PD-1)	CD27	CD4	CD8	CD3		Viabillity

Multicolor Immunophenotyping. Deep immune cell immunophenotyping was performed on human blood (A). Using a DURAClone IM T Cell panel, red outline, additional markers were added to increase the breadth of cell types (B). The analysis was completed on the CytoFLEX LX configured with 6 lasers. Fluorochromes were spread across different lasers to reduce spectral overlap. Using CytExpert software,sSequential hierarchical gating was used to identify B and T cell populations including deep analysis of the T cell compartment.

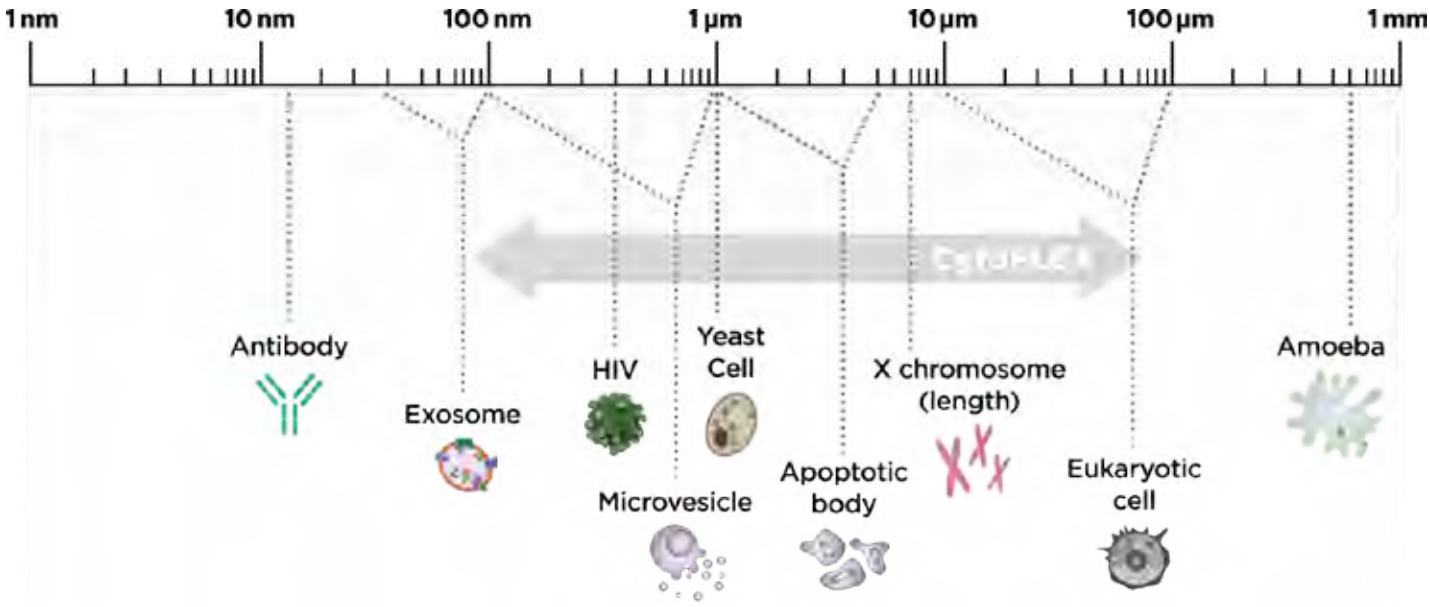


Dimensionality reduction using t-Distributed Stochastic Neighbor Embedding (tSNE). a) Pre-gating for doublet exclusion and the identification of viable CD3+ T-cells was performed on the same staining using Kaluza Analysis Software. The R Console plugin was used to perform tSNE analysis on pre-gated, compensated data and a new fcs file containing the tSNE parameters was generated. A population of interest (Pol) was gated on the tSNE plot. b) Expression patterns for CD8+ T-cells (blue) and the population of interest (red) were visulized for CD27 vs. CD28 and CD45RA vs. CCR7 using standard dot plots in Kaluza.

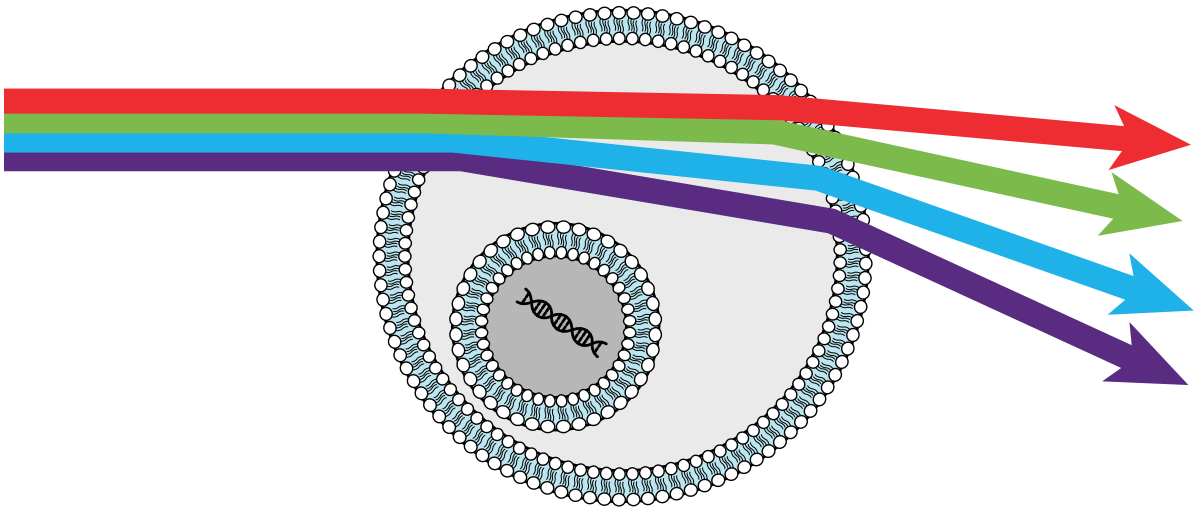
I enjoy the ability to swap out filters-that's a huge advantage of the instrument. I don't have to purchase additional filters, it already comes with all the filters that I would ever need. It also allows me to upgrade the instrument. Currently, I only have 2 lasers and I can upgrade to the violet laser, I can upgrade to a plate loader, I can upgrade to whatever I might need in the future, which is a huge advantage as a core manager.

Sarah Schuett, Core Lab Manager
North Carolina State Veterinary College

Nanoparticle Detection



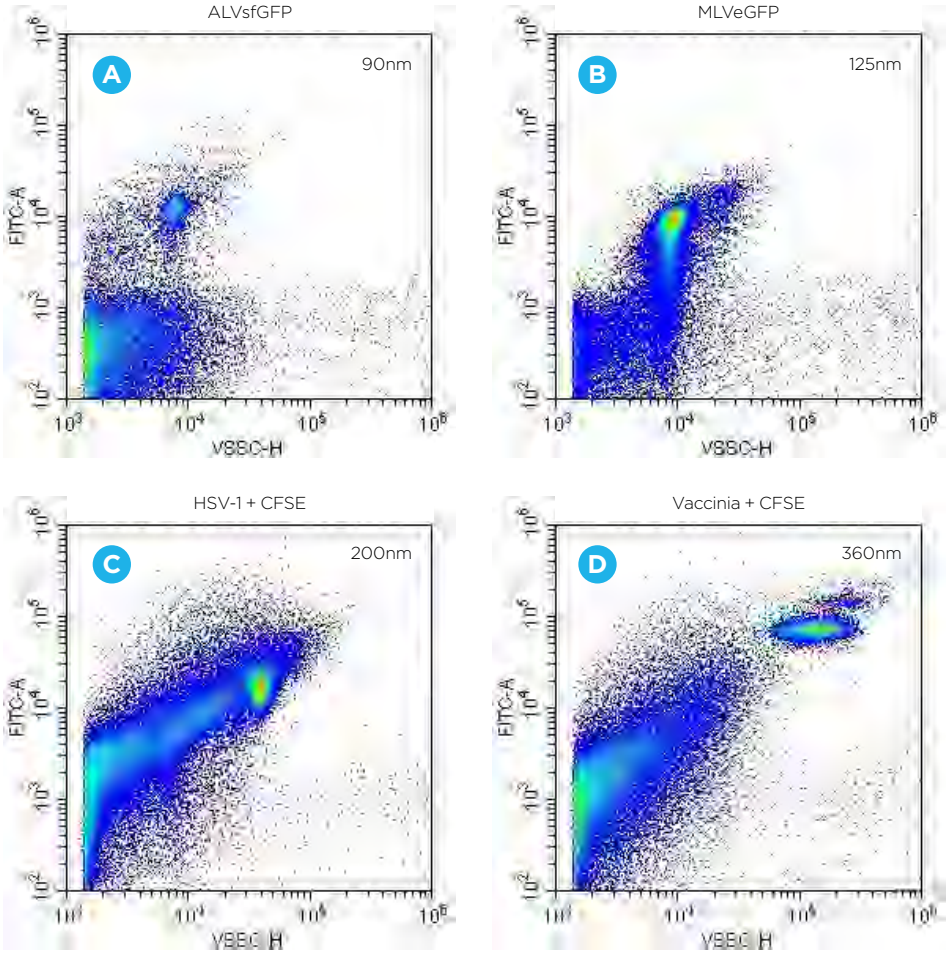
The advancement of flow cytometry into nanoparticle scale resolution, makes it possible to ask questions previously left to speculation. Several fundamental capabilities of flow cytometry make it an attractive platform for studying nanoparticles such as extracellular vesicles. That is the ability to detect large numbers of events, and discrimination of rare events, while simultaneously collecting information on phenotypic expression.



The CytoFLEX Platform of flow cytometers features the capability to measure side scatter off of the violet as well as the blue laser. This increases the range of particles that can be detected and analyzed within the sample. The smaller violet (405 nm) wavelength will result in more orthogonal light scattering at any given particle size than the blue (488 nm) wavelength.

The use of violet light will help to amplify the differences in the refractive indices between the particles and their surrounding media, and in turn increases the ability to detect particles with a lower refractive index, such as exosomes, microvesicles and silica nanoparticles.

The CytoFLEX Flow Cytometer has the resolution to detect 80 nm polystyrene particles. This facilitates analysis of biological nanoparticles within a phenotypic context.

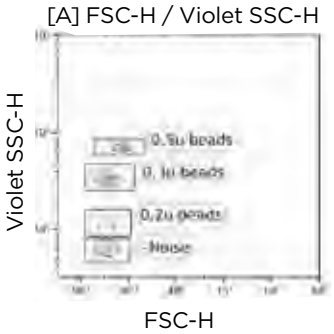


Analysis of fluorescently labeled enveloped viruses. (A) Avian leukosisvirus expressing superfolderGFP (ALVsfGFP). (B) Murine Leukemia Virus expressing eGFP(MLVeGFP). (C) HSV-1(TK-strain) and (D) Vaccinia (VVDD strain) labeled with the dye carboxyfluoresceinsuccinimidylester (CFSE). The diameter sizes for these viruses (top right of each panel) are as reported in literature and determined by electron microscopy.

Data kindly provided by Vera A. Tang, Ph.D. and Marc-André Langlois, Ph.D., University of Ottawa.

"The CytoFLEX is the first flow cytometer with an acceptable noise range on which we can clearly demonstrate detection of extracellular vesicles down to a size of 150 nm*. The potential to combine small particle analysis with the detection of up to 13 additional fluorescence parameters makes this cytometer an outstanding instrument for extracellular vesicle detection."

Andreas Spittler, MD, Associate Professor for Pathophysiology, Medical University of Vienna, Core Facility Flow Cytometry & Department of Surgery, Research Laboratories



In order to achieve detection smaller than 200 nm, modifications to the method and rigorous control of instrument set up and sample preparation are required. See Set-Up of the CytoFLEX for Extracellular Vesicle Measurement, Andreas Spittler.

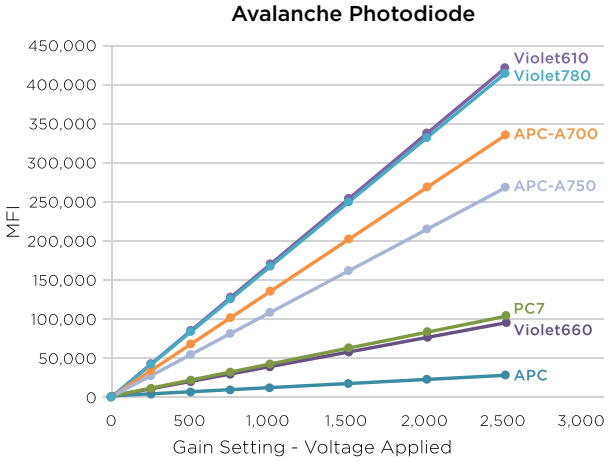
Multicolor Flow Cytometry Made Easy

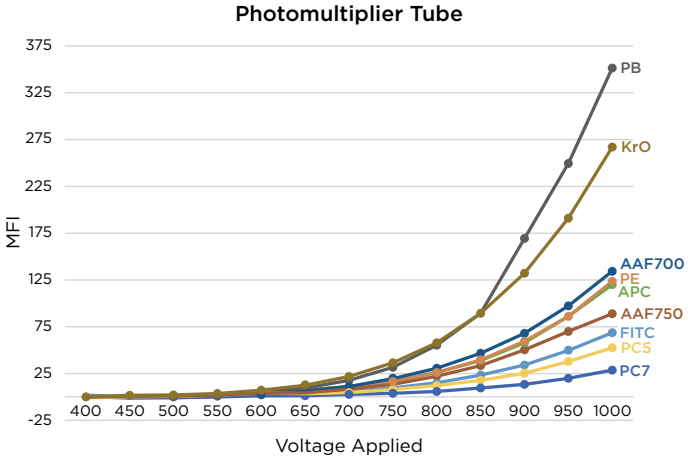
Novice to experienced flow cytometrists can quickly learn to operate the system, and can confidently generate and export publication quality data. The user interface uses common ribbon and contextual menus which makes instrument operation intuitive. The CytoFLEX workflow is a streamlined experience that allows you to focus on your sample.

Everyone likes to use the CytoFLEX because it's so easy. With complex panels there is flexibility to change channels and gains, which makes it easy to transfer setup from one cell type to another. This is based upon the ability to optimize a previously generated compensation matrix without rerunning the compensation. This saves money on antibodies and time because challenging experiments are easily set up in minutes instead of hours.

The CytoFLEX frees up time to do other things because it is reliable and people need very little guidance to use the instrument. Advanced analyses can be run by junior scientists. Time is key. We get really good data because of the performance and resolution of the system. This results in fewer failed experiments which is especially important when working with primary cells.

Anssi Kailaanmäki, Ph.D.
Head of Immunotherapy
Kuopio Center for Gene and Cell Therapy, FINLAND





The fluorescence intensities measured on the CytoFLEX Platform are linear to the corresponding detector gain settings. The software automatically recalculates spillover values in real time as the gains are adjusted. Due to the highly reproducible semiconductor process, the fluorescence intensities measured on the CytoFLEX Platform are linear to the corresponding detector gain settings. The non-linearity of the PMT based detection means that voltages need to be determined empirically.

Startup | Verify System Performance | Create Compensation | Startup Experiment | Shutdown

8 minutes | 1-2 minutes | 1-2 minutes

Our end-users have found the software very easy to learn and reliable. In our opinion, a researcher who has not done flow cytometry in the past typically needs about half the time to become proficient on CytoFLEX. We are very pleased with the wide dynamic range of the detectors and the capability of adjusting the gains of the detectors while seeing that the compensation matrix is recalculated virtually in real time. Although most users run panels with less than 15 colors, having an instrument capable of detecting the great majority of fluorochromes available on the market for flow cytometry assays is a major plus for a shared facility where we have many users with a great variety of applications.

After using the CytoFLEX LX in our lab several individual investigators at our institution either purchased or are in the process of purchasing CytoFLEX S cytometers for their exclusive use since their lab have a high volume of flow assays and they like the convenience of having a cytometer next to the bench where the actual samples are processed.

Florin Tuluc, MD, PhD
Flow Cytometry Core Laboratory
Children's Hospital of Philadelphia Research Institute, USA

Besides human-blood-multiparametric immunophenotyping and MV detection and characterization we routinely use our CytoFLEX for PI-cell cycle; calcium-production detection; mollusk-hemolymph-cell viability; dog/mouse immunophenotyping; mycobacterium/mycoplasma/ leishmania viability and counting analyses; and saliva immunophenotyping experiments.

In addition to the nanoscale scatter resolution of CytoFLEX, the instrument has a extreme-high-fluorescence sensitivity, allowing rare-event and low-expression-molecule detection, as well as a low consumption of monoclonal antibodies and reagents.

Alvaro Luiz Bertho, PhD
Senior Investigator and Vice-Head of Lab. of Immunoparasitology
Director of Flow Cytometry Core Facility
Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, BRAZIL

CytoFLEX Flow Cytometer

The CytoFLEX model provides the traditional laser palette and a number of channels to accommodate most basic flow cytometry assay needs.

Violet-Blue-Red (V-B-R) Series

The fully activated instrument includes five channels from the 488 nm (Blue) laser, three from the 638 nm (Red) laser, and five from the 405 nm (Violet) laser. The instrument includes 13 band pass filters which can be repositioned as needed. You can activate the lasers and detectors that you need now and add more channels later as your research needs grow. See the Configuration Table for a current list of available standard configurations.

Bandpass Filters

450/45	585/42	660/10 (2)	712/25
525/40 (2)	610/20 (2)	690/50	780/60 (3)

Standard Configurations

PART NUMBER	LASERS	FLUORESCENCE CHANNELS	405 NM VIOLET	488 NM BLUE	638 NM RED
B53000	3	13	**5**	**5**	**3**
B53001	3	12	**4**	**5**	**3**
B53002	3	12	**5**	**4**	**3**
B53003	3	11	**4**	**4**	**3**
B53004	3	11	**5**	**3**	**3**
B53006	3	10	**3**	**4**	**3**
B53005	3	10	**2**	**5**	**3**
B53037	2	10	**5**	**5**	
B53007	3	9	**3**	**3**	**3**
B53008	3	9	**2**	**4**	**3**
B96622	2	8		**5**	**3**
B53009	3	8	**2**	**3**	**3**
C02945	3	8	**2**	**4**	**2**
B53010	3	7	**2**	**3**	**2**
B53011	2	6		**3**	**3**
B53013	2	6		**4**	**2**
B53012	2	6	**3**	**3**	
C02944	2	6	**2**	**4**	
C02946	3	6	**2**	**2**	**2**
B53018	1	5		**5**	
B53014	2	5		**3**	**2**
B53019	1	4		**4**	
B53015	2	4		**3**	**1**
B53016	2	4		**2**	**2**
B53017	2	4	**2**	**2**	

VIOLET CHANNELS

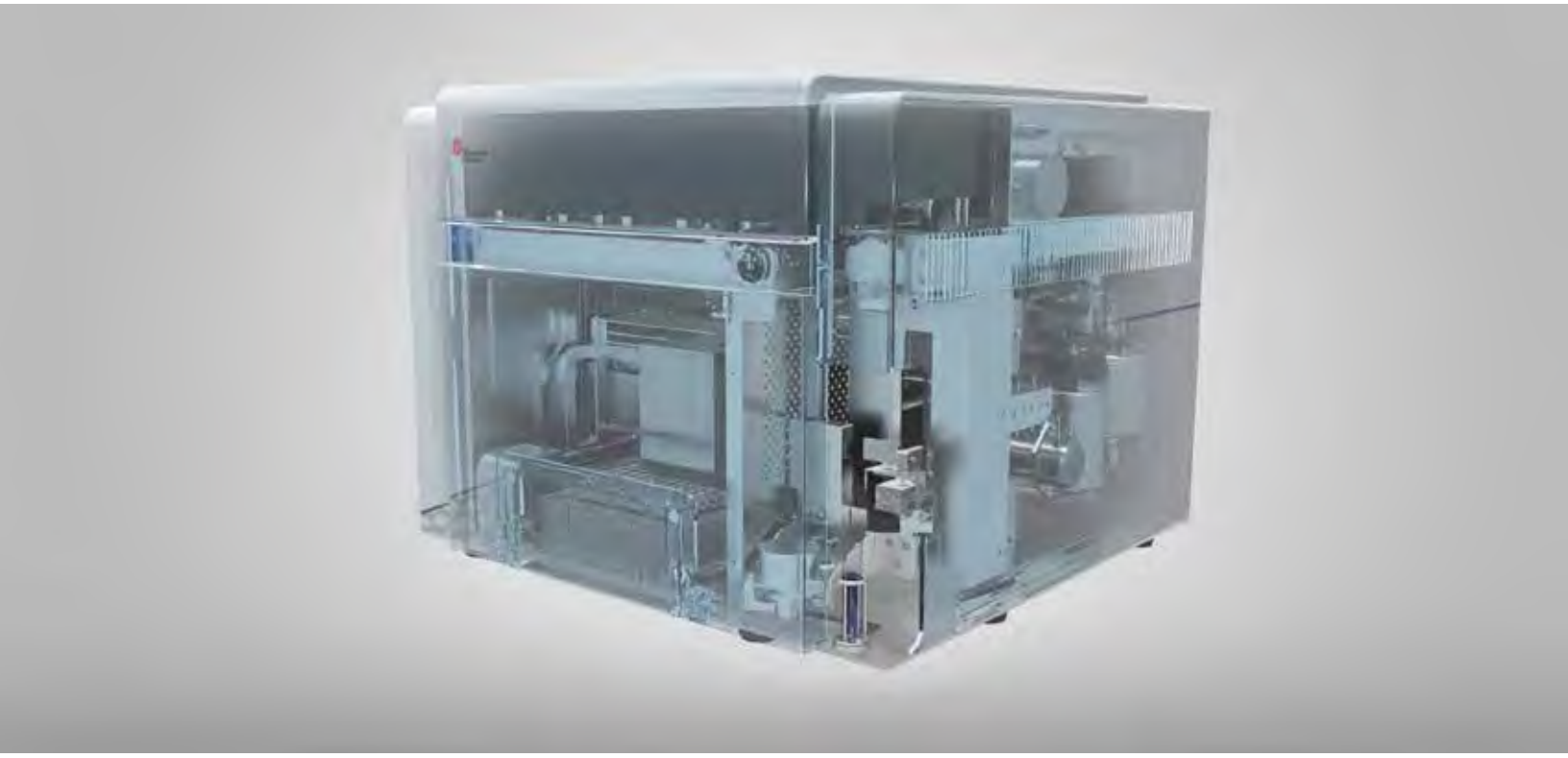
B690 B780 R660 R712 R780 V450

Excellent resolution of 8-speak SPHERO™ Rainbow Calibration Particles.

Plate Loader Options for the CytoFLEX Platform

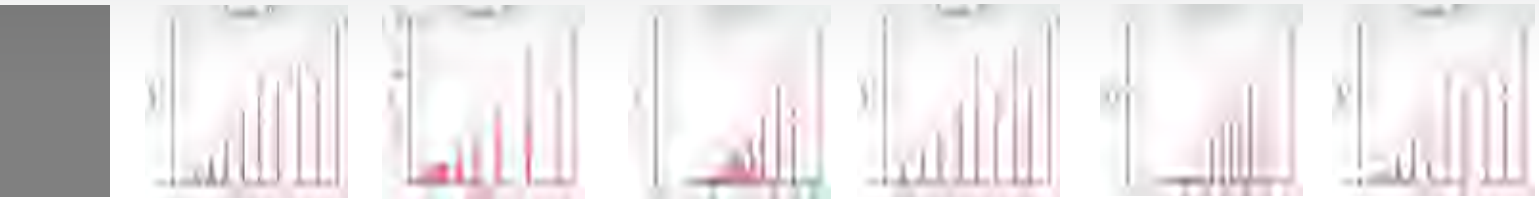
These optional accessories are compatible with all CytoFLEX platform models, CytoFLEX, CytoFLEX S and CytoFLEX LX. The sample loader fits inside of the instrument preserving the compact footprint and can be installed at any time. Three options are available depending on your needs.

Standard and Deep Well Plates with Tube/Plate Switch Control	Standard Plates with Tube/Plate Switch Control	Standard Plates with Manual Conversion between Tube/Plate Runs
Part Number C16574	Part Number C02396	Part Number B63215
Sample Injection Mode can be changed by using the Sample Injection Mode Control Switch.	Sample Injection Mode can be changed by using the Sample Injection Mode Control Switch.	Sample Injection Mode can be changed manually.
CytExpert version 2.2 or above	CytExpert version 2.0 or above	CytExpert version 1.1 or above



Inside the CytoFLEX. Transparent view of the CytoFLEX showing internal components of the fluidics and sample loading systems. The optional plate loader module is installed inside the main instrument preserving the overall compact footprint of the flow cytometer.

BLUE CHANNELS **RED CHANNELS**



CytoFLEX S Flow Cytometer

The CytoFLEX S models bring up to four laser instruments to the research community expanding the fluorochrome palette for special applications.

Violet-Blue-Yellow Green-Red (V-B-Y-R) Series

The fully activated instrument includes four fluorescent channels from the 405 nm (Violet) laser, two from the 488 nm (Blue) laser, four from the 561 nm (Yellow Green) laser, and three from the 638 nm (Red) laser. The instrument includes 13 band pass filters which can be repositioned as needed. You can activate the number of lasers and detectors that you need now and add more channels later as your research needs grow. See the Configuration Table for a current list of available standard configurations.

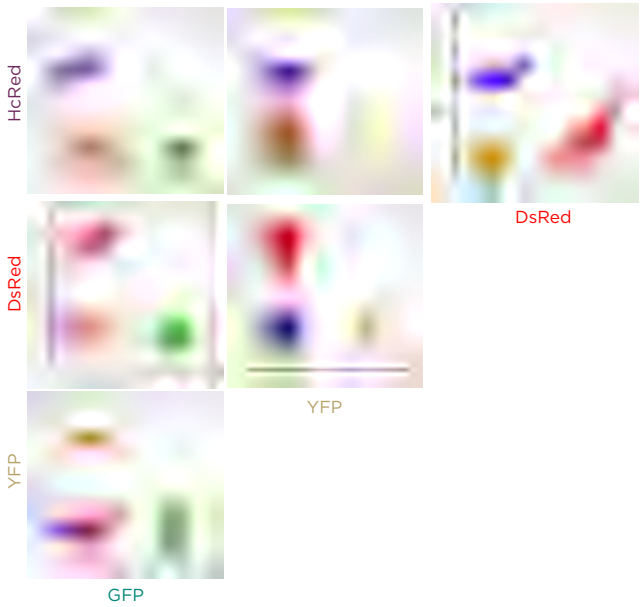
Includes 13 Repositionable Bandpass Filters

450/45	585/42	660/10 (2)	712/25
525/40 (2)	610/20 (2)	690/50 (2)	780/60 (2)

Standard Configurations

PART NUMBER	LASERS	FLUORESCENCE CHANNELS	405 NM VIOLET	488 NM BLUE	561 NM YELLOW GREEN	638 NM RED
B75408	4	13	**4**	**2**	**4**	**3**
B96620	3	10	**4**	**2**	**4**	
B75811	3	9		**2**	**4**	**3**
B96621	4	9	**2**	**2**	**3**	**2**
C02948	3	9	**4**	**2**		**3**
B75812	2	6		**2**	**4**	
C02947	3	6	**2**	**2**	**2**	

The **Yellow Green 561 nm** laser excites RFP and RFP derivatives such as DsRed and HcRed more efficiently than the **Blue 488 nm** laser. An additional benefit of spatially separated lasers is increased sensitivity, thus minimizing inter-laser compensation. Therefore, cells expressing GPF, YFP, DsRed, and HcRed, may be analyzed, resulting in superior resolution of simultaneously expressed multicolor fluorescent protein signals.



YELLOW GREEN CHANNELS

Excellent resolution of 8-speak SPHERO™ Rainbow Calibration Particles.

Near UV-Violet-Blue-Red (N-V-B-R) Series

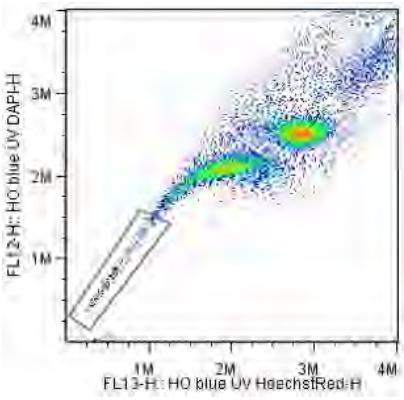
The fully activated instrument includes two fluorescent channels from the 375 nm (Near UV) laser, three from the 405 nm (Violet) laser, five from the 488 nm (Blue) laser, and three from the 638 nm (Red) laser. The instrument includes 13 band pass filters which can be repositioned as needed. The instrument has the capacity for 15 parameters, including 13 for fluorescence detection. You can activate the number of channels that you need now and add lasers and channels later as your research needs grow. See the Configuration Table for a list of available standard configurations.

Includes 13 Repositionable Bandpass Filters

450/45 (2)	585/42	660/10	690/50	780/60 (2)
525/40 (2)	610/20 (2)	675/30	712/25	

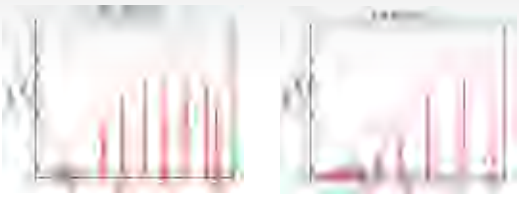
Standard Configurations

PART NUMBER	LASERS	FLUORESCENCE CHANNELS	375 NM NEAR UV	405 NM VIOLET	488 NM BLUE	638 NM RED
B78557	4	13	**2**	**3**	**5**	**3**
B78559	3	10	**2**		**5**	**3**
B78558	2	6	**2**		**4**	



The addition of the **375 nm near UV** laser, in a spatially separated discrete beam spot, enables excellent excitation of Hoescht, DAPI and brilliant UV dyes allowing for use of these dyes without incurring the cost of a 355 nm true UV laser. Dye Cycle Violet, while useful for performing side population analysis without a 355 nm laser, requires researchers to compromise on immunophenotyping as it spills over into the FITC and PE channels. Using the **375 nm** laser, researchers can go back to Hoescht for traditional side population analysis. Results are indistinguishable from data collected using a 355 nm laser.

NEAR UV CHANNELS



DAPI-A HoechstRed-A

Excellent resolution of 8-speak SPHERO™ Rainbow Calibration Particles.

CytoFLEX S Flow Cytometer

Violet-Blue-Red-Infrared (V-B-R-I) Series

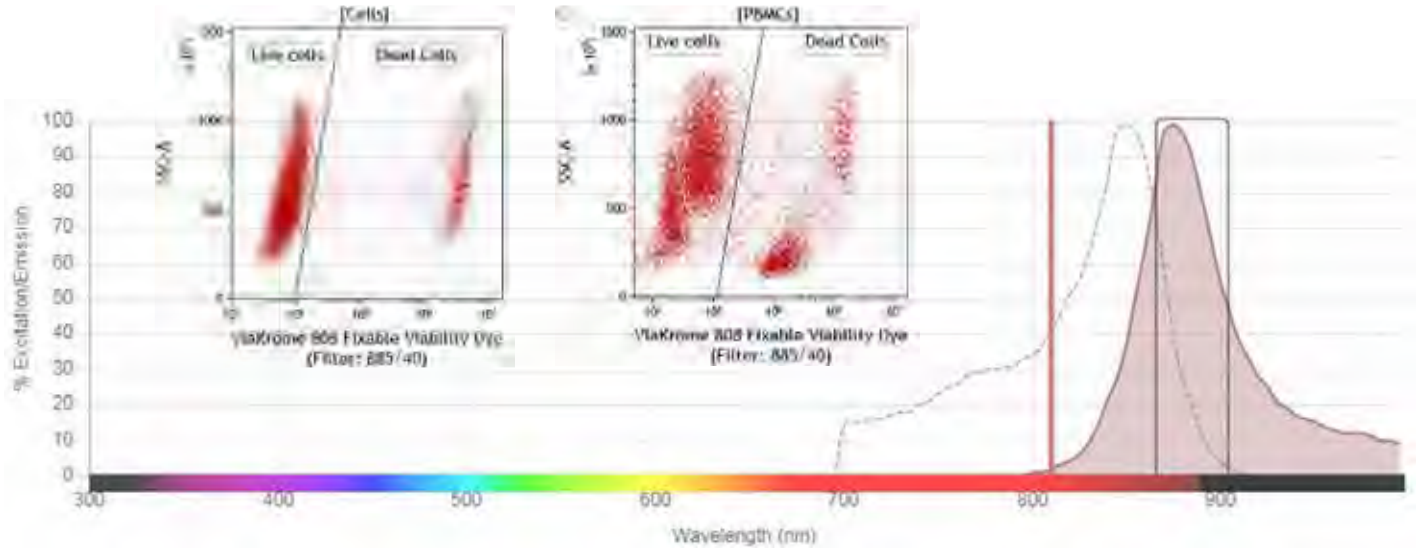
The fully activated instrument includes four fluorescent channels from the 405 nm (Violet) laser, four from the 488 nm (Blue) laser, three from the 638 nm (Red) laser, and two from the 808 nm (Infrared) laser. The instrument includes 13 band pass filters which can be repositioned as needed. You can activate the number of lasers and detectors that you need now and add more channels later as your research needs grow. See the Configuration Table for a current list of available standard configurations.

Includes 13 Repositionable Bandpass Filters

450/45	585/42	660/10 (2)	712/25	840/20
525/40 (2)	610/20	690/50	763/43 (2)	885/40

Standard Configurations

PART NUMBER	LASERS	FLUORESCENCE CHANNELS	405 NM VIOLET	488 NM BLUE	638 NM RED	808 NM INFRARED
C01161	4	13	**4**	**4**	**3**	**2**
C01160	3	10	**4**	**4**		**2**
C01159	3	9		**4**	**3**	**2**
C01158	2	6		**4**		**2**



Expanding the Usable Spectrum. ViaKrome 808 Fixable Viability dye excitation and emission spectrum, with 885/40 bandpass indicated. Plots show sample staining, Jurkat Cell Line (left) and PBMC (right).

INFRARED CHANNELS

AF790-A

PF840-A

Resolution of SPHERO™ Fluorescent IR Flow Cytometer Particles.

Near UV Violet Blue Yellow Green

The fully activated instrument includes two fluorescent channels from the 375 nm (Near UV) laser, two from the 488 nm (Blue) laser, four from the 405 nm (Violet) laser, and four from the 561 nm (Yellow Green) laser. The instrument includes 12 band pass filters which can be repositioned as needed. You can activate the number of lasers and detectors that you need now and add more channels later as your research needs grow. See the Configuration Table for a current list of available standard configurations.

Includes 13 Repositionable Bandpass Filters

450/45 (2)	585/42	660/10	690/50 (2)
525/40 (2)	610/20 (2)	675/30	780/60

Standard Configurations

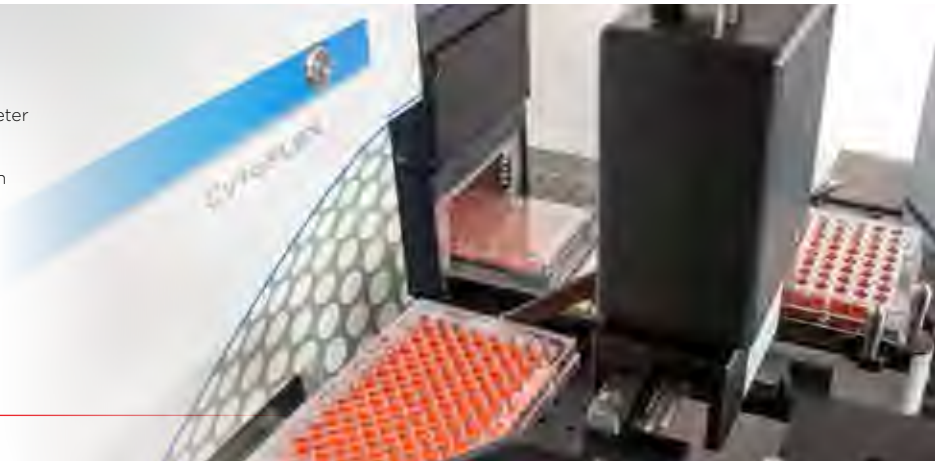
PART NUMBER	LASERS	FLUORESCENCE CHANNELS	375 NM NEAR UV	405 NM VIOLET	488 NM BLUE	561 NM YELLOW GREEN
B78560	4	12	2	4	2	4
B96619	3	10		4	2	4
B78561	3	8	2		2	4
B96618	2	6			2	4
C02949	2	4			2	2

For Even Higher Throughput Applications

Gain flexibility in your day by integrating your CytoFLEX Flow Cytometer to the Biomek i-Series Instruments for automated sample processing and data acquisition. Assay plates are transferred with the Biomek gripper directly to the CytoFLEX Flow Cytometer. Sample preparation [well] data, such as sample ID, is correlated with the information collected from the flow cytometer. Automate your complete cellular workflow with one trusted partner.

If you already have an automation solution, the CytExpert is an open platform. Our sales team can assist you in integrating the CytoFLEX Flow Cytometer based upon your workflow requirements.

Visit **biomek.beckman.com** to learn more about the i-Series



CytoFLEX LX Flow Cytometer

The CytoFLEX LX models bring configurations with up to six laser s and 21 fluorescent parameters to the research community.

Near UV-Violet-Blue-Yellow Green-Red-Infrared (N-V-B-Y-R-I) Series

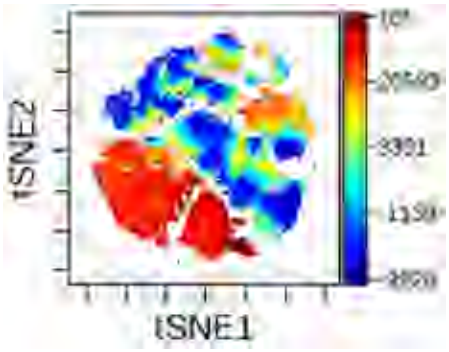
The fully activated instrument includes three fluorescent channels from the 355 nm (UV) laser, five from the 405 nm (Violet) laser, three from the 488 nm (Blue) laser, five from the 561 nm (Yellow Green) laser, three from the 638 nm (Red) laser, and two from the 808 nm (Infrared) laser. Instruments with as few as 14 fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key. The instrument includes 22 band pass filters which can be repositioned as needed. You can activate the number of lasers and detectors that you need now and add more channels later as your research needs grow. See the Configuration Table for a current list of available standard configurations.

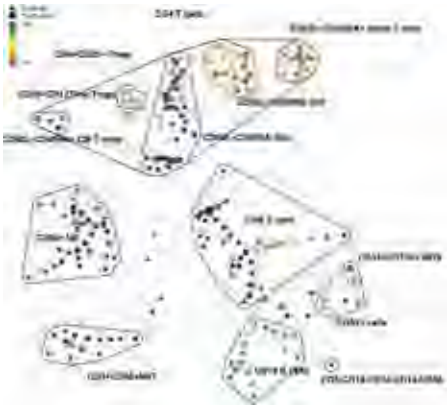
Includes 22 Repositionable Bandpass Filters

405/10	450/45 (2)	525/40 (3)	585/42	610/20 (3)	660/10 (2)	675/30 (2)
690/50	710/50	712/25	763/43 (3)	840/20	885/40	

Standard Configurations

PART NUMBER	LASERS	FLUORESCENCE CHANNELS	375 NM NEAR UV	405 NM VIOLET	488 NM BLUE	561 NM YELLOW GREEN	638 NM RED	808 NM INFRARED
C00445	6	21	**3**	**5**	**3**	**5**	**3**	**2**
C00446	5	19	**3**	**5**	**3**	**5**	**3**	**0**
C23009	4	16	**0**	**5**	**3**	**5**	**3**	**0**

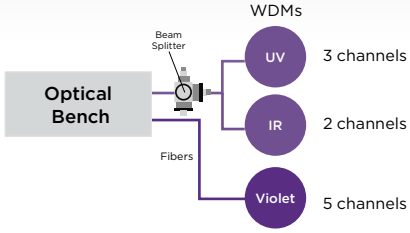




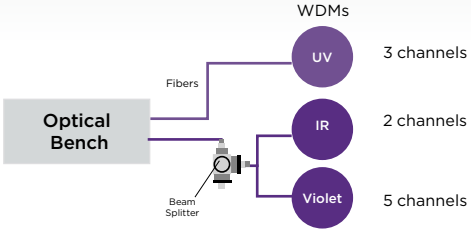
Immunophenotyping. Human whole blood was stained with 20-color panel and data acquired on a CytoFLEX LX UVBYRI flow cytometer. The data was prepared for supervised analysis using Kaluza Analysis software and then analyzed using viSNE, SPADE, and FlowSOM using Cytobank cloud-based platform at cytobank.org

Expand Violet, UV or Near UV channels with the CytoFLEX LX Beam Splitter

Addition of the CytoFLEX LX Beam Splitter allows the instrument to configure the IR detectors to detect emission from the UV, Near UV, or Violet WDM. The signal loss from splitting can be recovered by increasing the gain on the detectors.



UV or Near UV
3 channels → 5 channels



Violet
5 channels → 7 channels

UV-Violet-Blue-Yellow Green-Red-Infrared (U-V-B-Y-R-I) Series

The fully activated instrument includes three fluorescent channels from the 355 nm (UV) laser, five from the 405 nm (Violet) laser, three from the 488 nm (Blue) laser, five from the 561 nm (Yellow Green) laser, three from the 638 nm (Red) laser, and two from the 808 nm (Infrared) laser. The instrument includes 22 band pass filters which can be repositioned as needed. You can activate the number of lasers and detectors that you need now and add more channels later as your research needs grow. See the Configuration Table for a current list of available standard configurations.

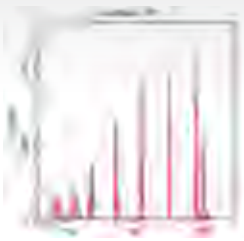
Includes 25 Repositionable Bandpass Filters

405/10	405/30	450/45	525/40 (3)	585/42	610/20 (3)	660/10 (2)	675/30 (2)
690/50	710/50	763/43 (3)	712/25	763/43 (3)	840/20	885/40	

Available Configurations

PART NUMBER	LASERS	FLUORESCENCE CHANNELS	355 NM UV	405 NM VIOLET	488 NM BLUE	561 NM YELLOW GREEN	638 NM RED	808 NM INFRARED
C11186	6	21	**3**	**5**	**3**	**5**	**3**	**2**
C11185	5	19	**3**	**5**	**3**	**5**	**3**	
C11183	4	14	**3**	**5**	**3**		**3**	
C11184	4	14	**3**	**5**	**3**	**3**		

UV CHANNELS



UV405-A

UV525-A

UV675-A

Accessories and Consumables

Start up kits are available to ensure that when your unit arrives you will be ready to start your experiments. We also offer kits and consumables for the routine use and maintenance. Each instrument contains standard band pass filters. We also offer a variety of non-standard filters for specialized applications.

Startup Kits* & Preventive Maintenance Kits

Part Number	Description
B55031	CytoFLEX Startup Reagents (tubes)
C14907	CytoFLEX Startup Reagents (plates)
C33328	CytoFLEX Startup Reagents (deep well plates)
C14908	CytoFLEX Startup Reagents (IR/tubes)
C14909	CytoFLEX Startup Reagents (IR/plates)
C33329	CytoFLEX Startup Reagents (IR/deep well plates)

Part Number	Description
C02943	Preventive Maintenance Kit
A04-1-0048	Peristaltic Sample Tubing Replacement Kit
A04-1-0041	Sheath Filter

*Includes daily QC, sheath fluid, FlowClean, Contrad, and sample tubes or plates

Consumables & Miscellaneous Replacement Parts

Part Number	Description
81911	Contrad 70
B53230	CytoFLEX Daily QC Fluorospheres
C06147	CytoFLEX Daily IR QC Fluorospheres
B51503	CytoFLEX Sheath Fluid
A64669	FlowClean Cleaning Agent
609844	Microtiter Plates, 96-well Flat Bottom
609801	Microtiter Plates, 96-well V Bottom
B63213	Plate Loader Sample Probe (with tubing to attach to plate assembly)

Part Number	Description
B71294	Sample Needle, 113 mm (orange bead)
A04-1-0034	Sample Needle, 115 mm (blue bead)
A04-1-0038	Deep Clean Solution Bottle Kits
A04-1-0036	Sheath Bottle Kit
A04-1-0037	Waste Bottle Kit
7547155	10 L Waste Tank
B86549	10 L Waste/Sheath Tanks Wiring Harness

Optional Bandpass Filters

Part Number	Description
A01-1-0048	405/10 nm Bandpass Filter
B99146	405/30 nm Bandpass Filter
A01-1-0049	450/45 nm Bandpass Filter
B90300	450/45 nm Bandpass with OD1 Filter
A01-1-0050	488/8 nm Bandpass Filter
B76128	510/20 nm Bandpass Filter
B90294	510/20 nm Bandpass with OD1 Filter
B76124	515/20 nm Bandpass Filter
A01-1-0051	525/40 nm Bandpass Filter
B90303	525/40 nm Bandpass with OD1 Filter
B76139	550/30 nm Bandpass Filter
B72627	561/6 nm Bandpass Filter
B76121	585/15 nm Bandpass Filter
B71089	585/30 nm Bandpass Filter
A01-1-0052	585/42 nm Bandpass Filter

Part Number	Description
B76117	595/20 nm Bandpass Filter
A01-1-0053	610/20 nm Bandpass Filter
B90297	610/20 nm Bandpass with OD1 Filter
A01-1-0054	638/6 nm Bandpass Filter
A01-1-0055	660/10 nm Bandpass Filter
B78244	675/30 nm Bandpass Filter
A01-1-0056	690/50 nm Bandpass Filter
B71092	710/50 nm Bandpass Filter
A01-1-0057	712/25 nm Bandpass Filter
B78217	740/35 nm Bandpass Filter
B99143	763/43 nm Bandpass Filter
A01-1-0058	780/60 nm Bandpass Filter
B78220	819/44 nm Bandpass Filter
B99144	840/20 nm Bandpass Filter
B99145	885/40 nm Bandpass Filter

Part Number	Description
C30171	Custom Optical Filter Holder (1) with Screws (2)
C30249	Custom Optical Filter Holder Mounting Fixture
C32857	Custom Optical Filter Holder Screws (2)

DURAClone Antibody Panels

Powered by
DURA Innovations
Dry Unitized Reagent Assays

Beckman Coulter offers expertly designed and optimized pre-formulated antibody panels using our DURA Innovation dry formulation technology. Each panel provides key markers for characterizing the specified cellular population and includes enough reagents for 25 tests. Depending on your CytoFLEX configuration you may extend the panels with additional markers of interest in liquid format.

405 NM		488 NM					638 NM					
450/45	525/40	525/40	585/42	610/20	690/50	780/60	660/10		712/25		780/60	
PB	KrO	FITC	PE	ECD	PC5.5	PC7	APC	AF647	AF700	APC-A700	APC-A750	AF750
DURAClone Immunophenotyping (IM)												
Basic Tube (Part Number B53309)												
-	CD45	CD16	CD56	CD19	-	CD14	CD4	-	CD8	-	CD3	-
B Cell Tube (Part Number B53318)												
IgM	CD45	IgD	CD21	CD19	-	CD27	CD24	-	-	-	CD38	-
T Cell Subsets Tube (Part Number B53328)												
CD57	CD45	CD45RA	CCR7	CD28	PD1	CD27	CD4	-	CD8	-	CD3	-
Dendritic Cells Tube (Part Number B53351)												
HLA-DR	CD45	CD16	Lin**	-	CD1c	CD11c	Clec9A	-	-	CD123	-	-
TCRs Tube (Part Number B53340)												
TCRVδ2	CD45	TCRγδ	TCRαβ	HLA-DR	-	TCRVδ1	CD4	-	CD8	-	CD3	-
Treg Tube (Part Number B53346)												
Helios	CD45	CD45RA	CD25	-	CD39	CD4	-	FoxP3	-	-	CD3	-
Granulocytes Tube (Part Number B88651)												
CD15	CD45	CD294	-	CD16	CD33	CD11b	PD-L1	-	-	Lin***	CD62L	-
Count Tube (Part Number C00162)												
-	-	CD45	Counting Beads	-	7-AAD	-	-	-	-	-	-	-
DURAClone Immune Function (IF)												
T Activation (Part Number B88649)												
CD4	-	IFNγ	TNFα	-	-	IL-2	-	-	CD8	-	-	CD3
T Helper Cell (Part Number C04666)												
IL-17A	-	IFNγ	-	-	-	IL-4	CD4	-	-	-	-	CD3
If Monocytes Activation C21858 (25 tests PUO)												
CD14	CD45	-	HLA-DR	-	-	-	-	-	-	TNFα	-	-
If Basophil Activation C23406 (25 tests PUO)												
CD63	CD45	-	CD203c	-	-	CD3	-	CD294	-	-	-	-
DURAClone Rare Event (RE)												
CLB Tube (Part Number B80393)												
CD20	CD45	CD81	ROR-1	-	CD79b	CD19	CD5	-	-	-	CD43	-
PC Tube (Part Number B80394)												
CD38	CD45	CD81	CD27	-	CD19	CD200	CD138	-	-	-	CD56	-
ALB Tube (Part Number C00163)												
-	CD45	CD58	-	CD34	CD10	CD19	-	-	-	CD38	CD20	-

** CD3/CD14/CD19/CD20/CD56 | *** CD3/14/CD19/CD56

DURActive

Part Number	Description
C11101	DURActive 1 (PMA, Ionomycin, Brefeldin A)
C11102	DURActive 2 (PMA, Ionomycin)
C21857	DURACtive 3 (LPS, Brefeldin A)

Part Number	Description
C36614	ViaKrome 405 Fixable Viability Dye
C36620	ViaKrome 561 Fixable Viability Dye
C36624	ViaKrome 638 Fixable Viability Dye
C36628	ViaKrome 808 Fixable Viability Dye

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For over 80 years Beckman Coulter has driven innovation. We remain committed to shaping flow cytometry technology to fit seamlessly into your lab's workflow and to provide an optimal user experience. When you choose a Beckman Coulter instrument you receive the highest level of expertise, innovation, and quality assurance.

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BECKMAN COULTER
Life Sciences

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FLOW-5237SB12.19

EXHIBIT 12

BECKMAN COULTER
Life Sciences

CytoFLEX Flow Cytometer

Violet-Blue-Red (V-B-R) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 15 parameters, including 13 for fluorescence detection. The fully activated instrument includes five channels from the 405 nm (Violet) laser, five from the 488 nm (Blue) laser, and three from the 638 nm (Red) laser. Instruments with as few as four fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Red	638 nm	50 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 13 repositionable filters

450/45	660/10 (2)
525/40 (2)	690/50
585/42	712/25
610/20 (2)	780/60 (3)

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission bandpass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channels off of the 405, 488, and 638 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC):
80 nm polystyrene particles

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: < 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving 3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

ULTRA-LOW PRESSURE PERISTALTIC SHEATH AND SAMPLE DELIVERY SYSTEM

Low maintenance system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

Gravimetric calibration for absolute counts within CytExpert Software.

FLUID CAPACITY

Standard 4 L tanks

Optional 10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

96-well Standard Flat, U and V bottom plates, and 96-deep well plates. Refer to CytoFLEX Plate Loaders Technical Specification Sheet _FLOW-3308SPEC12.17 for details on all plate loader options.

DATA MANAGEMENT

SOFTWARE

The CytExpert software is a full-feature software package that controls instrument operation, data collection and analysis.

Three different installation modes are available depending on the level of security required.

The Default installation requires no user login.

For multiuser instruments, the User Management mode requires user login and contains features for user and role management.

Electronic Records Management installation provides tools that facilitate compliance with 21 CFR Part 11, Electronic Records and Electronic Signatures.

An API (Application Programming Interface) is available and allows external software to perform operations such as running methods and for basic control of the plate loader.

If desired, export FCS files for offline analysis in Kaluza, FCSExpress, FlowJo, and other platforms.

STANDARDIZATION

Daily QC beads or any other reference material that is relevant for your application may be used as the standardization sample to set target values and calibrate the gain settings automatically.

LANGUAGE

English and Chinese

OPERATING SYSTEM

Windows® 7 Professional 64-bit

Windows® 8 Professional 64-bit

Windows® 10 Professional 64-bit

FCS FORMAT

FCS 3.0

MINIMUM COMPUTER SPECIFICATIONS

CPU: Intel® I3 @ 2.9 GHz 1 Gigabit Ethernet port

RAM: 4 GB 2 USB 3.0 ports

Storage: 256 GB 4 USB 2.0 ports

COMPENSATION

Automatic full matrix compensation

Manual full matrix compensation

Novel Compensation Library: store fluorescent spillover values of dyes to easily determine the correct compensation matrix with new gain settings

Import/export compensation values between experiments

Absolute linear gain amplification enables the use of compensation settings between experiments and sample types

INSTALLATION

DIMENSIONS (W X D X H)

Cytometer (with or without Plate Loader)

42.5 cm x 42.5 cm x 34 cm

16.7 in x 16.7 in x 13.4 in

Tanks and Holder

14 cm x 35.6 cm x 35.6 cm

5.5 in x 14.0 in x 14.0 in

WEIGHT

Cytometer: 23.4 kg / 51.6 lbs

Cytometer with Plate Loader: 28 kgs / 61.7 lbs

POWER SPECIFICATIONS

Voltage: 100–240 V Power: 150 -250 W

OPERATING TEMPERATURE NON-CONDENSING

15-27 °C, 59-80.6 °F

BECKMAN COULTER
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For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

FLOW-4648SPEC12.18

EXHIBIT 13

BECKMAN COULTER
Life Sciences

CytoFLEX S Flow Cytometer

Near UV-Violet-Blue-Yellow Green (N-V-B-Y) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 14 parameters, including 12 for fluorescence detection. The fully activated instrument includes two fluorescent channels from the 375 nm (Near UV) laser, four from the 405 nm (Violet) laser, two from the 488 nm (Blue) laser, and four from the 561 nm (Yellow Green) laser. Instruments with as few as four fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
Near UV	375 nm	60 mW
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Yellow Green	561 nm	30 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 13 repositionable filters

450/45 (2)	660/10
525/40 (2)	675/30
585/42	690/50 (2)
610/20 (2)	780/60

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission band pass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channels off of the 405, 488, and 561 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%. For detection channels off of the 375 nm laser, the criteria is ≤ 7.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC):
80 nm polystyrene particles

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: < 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving 3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

ULTRA-LOW PRESSURE PERISTALTIC SHEATH AND SAMPLE DELIVERY SYSTEM

Low maintenance system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

Gravimetric calibration for absolute counts within CytExpert Software.

FLUID CAPACITY

Standard 4 L tanks

Optional 10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

96-well Standard Flat, U and V bottom plates, and 96-deep well plates. Refer to CytoFLEX Plate Loaders Technical Specification Sheet _FLOW-3308SPEC12.17 for details on all plate loader options.

DATA MANAGEMENT

SOFTWARE

The CytExpert software is a full-feature software package that controls the instrument's operation, collection of experiment data, and analysis of the results.

Three different installation modes are available, depending on the level of security required.

The Default installation requires no user login.

For multiuser instruments, the User Management mode requires user login and contains features for user and role management.

Electronic Records Management installation provides tools that facilitate compliance with 21 CFR Part 11, Electronic Records and Electronic Signatures.

An API (Application Programming Interface) is available and allows external software to perform operations such as running methods and for basic control of the plate loader.

If desired, export FCS files for offline analysis in Kaluza, FCSExpress, FlowJo, and other platforms.

STANDARDIZATION

Daily QC beads or any other reference material that is relevant for your application may be used as the standardization sample to set target values and calibrate the gain settings automatically.

LANGUAGE

English and Chinese

OPERATING SYSTEM

Windows® 7 Professional 64-bit

Windows® 8 Professional 64-bit

Windows® 10 Professional 64-bit

FCS FORMAT

FCS 3.0

MINIMUM COMPUTER SPECIFICATIONS

CPU: Intel® I3 @ 2.9 GHz 1 Gigabit Ethernet port

RAM: 4 GB 2 USB 3.0 ports

Storage: 256 GB 4 USB 2.0 ports

COMPENSATION

Automatic full matrix compensation

Manual full matrix compensation

Novel Compensation Library: store fluorescent spillover values of dyes to easily determine the correct compensation matrix with new gain settings

Import/export compensation values between experiments

Absolute linear gain amplification enables the use of compensation settings between experiments and sample types

INSTALLATION

DIMENSIONS (W X D X H)

Cytometer (with or without Plate Loader)

42.5 cm x 42.5 cm x 34 cm

16.7 in x 16.7 in x 13.4 in

Tanks and Holder

14 cm x 35.6 cm x 35.6 cm

5.5 in x 14.0 in x 14.0 in

WEIGHT

Cytometer: 23.4 kg / 51.6 lbs

Cytometer with Plate Loader: 28 kgs / 61.7 lbs

POWER SPECIFICATIONS

Voltage: 100–240 V Power: 150 -250 W

OPERATING TEMPERATURE NON-CONDENSING

15-27 °C, 59-80.6 °F

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FLOW-4652SPEC12.18

EXHIBIT 14

BECKMAN COULTER
Life Sciences

CytoFLEX S Flow Cytometer

Near UV-Violet-Blue-Red

(N-V-B-R) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 15 parameters, including 13 for fluorescence detection. The fully activated instrument includes two fluorescent channels from the 375 nm (Near UV) laser, three from the 405 nm (Violet) laser, five from the 488 nm (Blue) laser, and three from the 638 nm (Red) laser. Instruments with as few as six fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
Near UV	375 nm	60 mW
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Red	638 nm	50 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 13 repositionable filters

450/45 (2)	675/30
525/40 (2)	690/50
585/42	712/25
610/20 (2)	780/60/2
660/10	

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission band pass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channels off of the 405, 488, and 561 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%. For detection channels off of the 375 nm laser, the criteria is ≤ 7.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC):
80 nm polystyrene particles.

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: < 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving 3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

ULTRA-LOW PRESSURE PERISTALTIC SHEATH AND SAMPLE DELIVERY SYSTEM

Low maintenance system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

Gravimetric calibration for absolute counts within CytExpert Software.

FLUID CAPACITY

Standard 4 L tanks

Optional 10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

96-well Standard Flat, U and V bottom plates, and 96-deep well plates. Refer to CytoFLEX Plate Loaders Technical Specification Sheet _FLOW-3308SPEC12.17 for details on all plate loader options.

DATA MANAGEMENT

SOFTWARE

The CytExpert software is a full-feature software package that controls the instrument's operation, collection of experiment data, and analysis of the results.

Three different installation modes are available, depending on the level of security required.

The Default installation requires no user login.

For multiuser instruments, the User Management mode requires user login and contains features for user and role management.

Electronic Records Management installation provides tools that facilitate compliance with 21 CFR Part 11, Electronic Records and Electronic Signatures.

An API (Application Programming Interface) is available and allows external software to perform operations such as running methods and for basic control of the plate loader.

If desired, export FCS files for offline analysis in Kaluza, FCSExpress, FlowJo, and other platforms.

STANDARDIZATION

Daily QC beads or any other reference material that is relevant for your application may be used as the standardization sample to set target values and calibrate the gain settings automatically.

LANGUAGE

English and Chinese

OPERATING SYSTEM

Windows® 7 Professional 64-bit

Windows® 8 Professional 64-bit

Windows® 10 Professional 64-bit

FCS FORMAT

FCS 3.0

MINIMUM COMPUTER SPECIFICATIONS

CPU: Intel® I3 @ 2.9 GHz 1 Gigabit Ethernet port

RAM: 4 GB 2 USB 3.0 ports

Storage: 256 GB 4 USB 2.0 ports

COMPENSATION

Automatic full matrix compensation

Manual full matrix compensation

Novel Compensation Library: store fluorescent spillover values of dyes to easily determine the correct compensation matrix with new gain settings

Import/export compensation values between experiments

Absolute linear gain amplification enables the use of compensation settings between experiments and sample types

INSTALLATION

DIMENSIONS (W X D X H)

Cytometer (with or without Plate Loader)

42.5 cm x 42.5 cm x 34 cm

16.7 in x 16.7 in x 13.4 in

Tanks and Holder

14 cm x 35.6 cm x 35.6 cm

5.5 in x 14.0 in x 14.0 in

WEIGHT

Cytometer: 23.4 kg / 51.6 lbs

Cytometer with Plate Loader: 28 kgs / 61.7 lbs

POWER SPECIFICATIONS

Voltage: 100–240 V Power: 150 -250 W

OPERATING TEMPERATURE NON-CONDENSING

15-27 °C, 59-80.6 °F

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FLOW-4653SPEC12.18

EXHIBIT 15

BECKMAN COULTER
Life Sciences

CytoFLEX S Flow Cytometer

Violet-Blue-Yellow Green-Red (V-B-Y-R) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 15 parameters, including 13 for fluorescence detection. The fully activated instrument includes four channels from the 405 nm (Violet) laser, two from the 488 nm (Blue) laser, four from the 561 nm (Yellow Green) laser and three from the 638 nm (Red) laser. Instruments with as few as six fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Yellow Green	561 nm	30 mW
Red	638 nm	50 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 13 repositionable filters

450/45	660/10 (2)
525/40 (2)	690/50
585/42	712/25
610/20 (2)	780/60 (3)

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission bandpass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channels off of the 405, 488, 561 and 638 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC): 80 nm polystyrene particles

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: < 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving 3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

ULTRA-LOW PRESSURE PERISTALTIC SHEATH AND SAMPLE DELIVERY SYSTEM

Low maintenance system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

Gravimetric calibration for absolute counts within CytExpert Software.

FLUID CAPACITY

Standard 4 L tanks

Optional 10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

96-well Standard Flat, U and V bottom plates, and 96-deep well plates. Refer to CytoFLEX Plate Loaders Technical Specification Sheet _FLOW-3308SPEC12.17 for details on all plate loader options.

DATA MANAGEMENT

SOFTWARE

The CytExpert software is a full-feature software package that controls instrument operation, data collection and analysis.

Three different installation modes are available depending on the level of security required.

The Default installation requires no user login.

For multiuser instruments, the User Management mode requires user login and contains features for user and role management.

Electronic Records Management installation provides tools that facilitate compliance with 21 CFR Part 11, Electronic Records and Electronic Signatures.

An API (Application Programming Interface) is available and allows external software to perform operations such as running methods and for basic control of the plate loader.

If desired, export FCS files for offline analysis in Kaluza, FCSExpress, FlowJo, and other platforms.

STANDARDIZATION

Daily QC beads or any other reference material that is relevant for your application may be used as the standardization sample to set target values and calibrate the gain settings automatically.

LANGUAGE

English and Chinese

OPERATING SYSTEM

Windows® 7 Professional 64-bit

Windows® 8 Professional 64-bit

Windows® 10 Professional 64-bit

FCS FORMAT

FCS 3.0

MINIMUM COMPUTER SPECIFICATIONS

CPU: Intel® I3 @ 2.9 GHz 1 Gigabit Ethernet port

RAM: 4 GB 2 USB 3.0 ports

Storage: 256 GB 4 USB 2.0 ports

COMPENSATION

Automatic full matrix compensation

Manual full matrix compensation

Novel Compensation Library: store fluorescent spillover values of dyes to easily determine the correct compensation matrix with new gain settings

Import/export compensation values between experiments

Absolute linear gain amplification enables the use of compensation settings between experiments and sample types

INSTALLATION

DIMENSIONS (W X D X H)

Cytometer (with or without Plate Loader)

42.5 cm x 42.5 cm x 34 cm

16.7 in x 16.7 in x 13.4 in

Tanks and Holder

14 cm x 35.6 cm x 35.6 cm

5.5 in x 14.0 in x 14.0 in

WEIGHT

Cytometer: 23.4 kg / 51.6 lbs

Cytometer with Plate Loader: 28 kgs / 61.7 lbs

POWER SPECIFICATIONS

Voltage: 100–240 V Power: 150 -250 W

OPERATING TEMPERATURE NON-CONDENSING

15-27 °C, 59-80.6 °F

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FLOW-4650SPEC12.18

EXHIBIT 16

BECKMAN COULTER
Life Sciences

CytoFLEX S Flow Cytometer

Violet-Blue-Red-Infrared (V-B-R-I) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 15 parameters, including 13 for fluorescence detection. The fully activated instrument includes four fluorescent channels from the 405 nm (Violet) laser, four from the 488 nm (Blue) laser, three from the 638 nm (Red) laser, and two from the 808 nm (Infrared) laser. Instruments with as few as six fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Red	638 nm	50 mW
Infrared	808 nm	60 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 13 repositionable filters

450/45	690/50
525/40 (2)	712/25
585/42	763/43 (2)
610/20	840/20
660/10 (2)	885/40

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission band pass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channels off of the 405, 488, and 561 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%. For detection channels off of the 375 nm laser, the criteria is ≤ 7.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC):
80 nm polystyrene particles

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: < 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving 3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

ULTRA-LOW PRESSURE PERISTALTIC SHEATH AND SAMPLE DELIVERY SYSTEM

Low maintenance system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

Gravimetric calibration for absolute counts within CytExpert Software.

FLUID CAPACITY

Standard 4 L tanks

Optional 10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

96-well Standard Flat, U and V bottom plates, and 96-deep well plates. Refer to CytoFLEX Plate Loaders Technical Specification Sheet _FLOW-3308SPEC12.17 for details on all plate loader options.

DATA MANAGEMENT

SOFTWARE

The CytExpert software is a full-feature software package that controls instrument operation, data collection, and analysis.

Three different installation modes are available, depending on the level of security required.

The Default installation requires no user login.

For multiuser instruments, the User Management mode requires user login and contains features for user and role management.

Electronic Records Management installation provides tools that facilitate compliance with 21 CFR Part 11, Electronic Records and Electronic Signatures.

An API (Application Programming Interface) is available and allows external software to perform operations such as running methods and for basic control of the plate loader.

If desired, export FCS files for offline analysis in Kaluza, FCSExpress, FlowJo, and other platforms.

STANDARDIZATION

Daily QC beads or any other reference material that is relevant for your application may be used as the standardization sample to set target values and calibrate the gain settings automatically.

LANGUAGE

English and Chinese

OPERATING SYSTEM

Windows® 7 Professional 64-bit

Windows® 8 Professional 64-bit

Windows® 10 Professional 64-bit

FCS FORMAT

FCS 3.0

MINIMUM COMPUTER SPECIFICATIONS

CPU: Intel® I3 @ 2.9 GHz 1 Gigabit Ethernet port

RAM: 4 GB 2 USB 3.0 ports

Storage: 256 GB 4 USB 2.0 ports

COMPENSATION

Automatic full matrix compensation

Manual full matrix compensation

Novel Compensation Library: store fluorescent spillover values of dyes to easily determine the correct compensation matrix with new gain settings

Import/export compensation values between experiments

Absolute linear gain amplification enables the use of compensation settings between experiments and sample types

INSTALLATION

DIMENSIONS (W X D X H)

Cytometer (with or without Plate Loader)

42.5 cm x 42.5 cm x 34 cm

16.7 in x 16.7 in x 13.4 in

Tanks and Holder

14 cm x 35.6 cm x 35.6 cm

5.5 in x 14.0 in x 14.0 in

WEIGHT

Cytometer: 23.4 kg / 51.6 lbs

Cytometer with Plate Loader: 28 kgs / 61.7 lbs

POWER SPECIFICATIONS

Voltage: 100–240 V Power: 150 -250 W

OPERATING TEMPERATURE NON-CONDENSING

15-27 °C, 59-80.6 °F

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FLOW-4651SPEC12.18

EXHIBIT 17

BECKMAN COULTER
Life Sciences

CytoFLEX LX Flow Cytometer

UV-Violet-Blue-Yellow Green-Red-Infrared (U-V-B-Y-R-I) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 23 parameters, including 21 for fluorescence detection. The fully activated instrument includes three fluorescent channels from the 355 nm (UV) laser, five from the 405 nm (Violet) laser, three from the 488 nm (Blue) laser, five from the 561 nm (Yellow Green) laser, three from the 638 nm (Red) laser, and two from the 808 nm (Infrared) laser. Instruments with as few as 14 fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
UV	355 nm	20 mW
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Yellow Green	561 nm	30 mW
Red	638 nm	50 mW
Infrared	808 nm	60 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 23 repositionable filters

405/10	675/30 (2)
405/30	690/50
450/45 (2)	710/50
525/40 (3)	712/25
585/42	763/43 (3)
610/20 (3)	840/20
660/10 (2)	885/40

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission band pass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channel off of the 405, 488, 561, and 638 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%. For detection channels off of the 355 nm and 808 nm laser, the criteria is ≤ 7.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC): 80 nm polystyrene particles

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: ≤ 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving <3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

Ultra-low pressure peristaltic sheath and sample delivery system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

FLUID CAPACITY

10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

96-well Standard Flat, U and V bottom plates, and 96-deep well plates. Refer to CytoFLEX Plate Loaders Technical Specification Sheet for details on all plate loader options.

DATA MANAGEMENT

SOFTWARE

The CytExpert software is a full-feature software package that controls the instrument's operation, collection of experiment data, and analysis of the results.

Three different installation modes are available, depending on the level of security required.

The Default installation requires no user login.

For multiuser instruments, the User Management mode requires user login and contains features for role management.

Electronic Records Management installation provides tools that facilitate compliance with 21 CFR Part 11, Electronic Records and Electronic Signatures.

Gravimetric calibration for absolute counts within CytExpert Software.

An API (Application Programming Interface) is available and allows external software to perform operations such as running methods and for basic control of the plate loader.

If desired, export FCS files for offline analysis in Kaluza, FCSExpress, FlowJo, and other platforms.

STANDARDIZATION

Daily QC beads or any other reference material that is relevant for your application may be used as the standardization sample to set target values and calibrate the gain settings automatically.

LANGUAGE

English and Chinese

OPERATING SYSTEM

Windows® 7 Professional 64-bit

Windows® 8 Professional 64-bit

Windows® 10 Professional 64-bit

FCS FORMAT

FCS 3.0

MINIMUM COMPUTER SPECIFICATIONS

CPU: Intel Core i7, up to 3.9 GHz

RAM: 8 GB, 5 USB 2.0 and above ports

Storage: 1 TB drive in RAID1

Ethernet: Integrated 10M/100M/1000M GB

COMPENSATION

Automatic full matrix compensation

Manual full matrix compensation

Novel Compensation Library: store fluorescent spillover values of dyes to easily determine the correct compensation matrix with new gain settings

Import/export compensation values between experiments

Absolute linear gain amplification enables the use of compensation settings between experiments and sample types

INSTALLATION

DIMENSIONS (W X D X H)

Cytometer (with or without Plate Loader)

60.5 cm x 73.3 cm x 45.1 cm

Tanks and Holder

25 cm x 25 cm x 25 cm

WEIGHT

Cytometer: 79 kg

Cytometer with Plate Loader: 83.6 kg

POWER SPECIFICATIONS

Voltage: 100–240 V Power: 150 -250 W

OPERATING TEMPERATURE NON-CONDENSING

15-30 °C

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EXHIBIT 18

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CytoFLEX LX Flow Cytometer

Near UV-Violet-Blue-Yellow Green-Red-Infrared (N-V-B-Y-R-I) Series

OPTICS

EXCITATION OPTICS

The instrument has the capacity for 23 parameters, including 21 for fluorescence detection. The fully activated instrument includes three fluorescent channels from the 375 nm (Near UV) laser, five from the 405 nm (Violet) laser, three from the 488 nm (Blue) laser, five from the 561 nm (Yellow Green) laser, three from the 638 nm (Red) laser, and two from the 808 nm (Infrared) laser. Instruments with as few as 16 fluorescent channels activated are available with the ability to activate additional parameters as needed by purchasing an activation key.

LASER SPECIFICATIONS

Spatially Separated Laser Options (Beam Spot Size: 5 µm x 80 µm)

Laser	Wavelength	Power
Near UV	375 nm	60 mW
Violet	405 nm	80 mW
Blue	488 nm	50 mW
Yellow Green	561 nm	30 mW
Red	638 nm	50 mW
Infrared	808 nm	60 mW

FLOW CELL

Fixed integrated optics and quartz flow cell design with >1.3 numerical aperture.

Flow Cell dimensions: 430 µm x 180 µm internal diameter

FORWARD SCATTER DETECTION

Proprietary Homodyne FSC sensor system using silicon photodiodes with built in 488/8 nm bandpass filter.

BANDPASS FILTERS

Includes 22 repositionable filters

405/10	690/50
450/45 (2)	710/50
525/40 (3)	712/25
585/42	763/43 (3)
610/20 (3)	840/20
660/10 (2)	885/40
675/30 (2)	

FLUORESCENCE AND SIDE SCATTER DETECTION

Fluorescence and side scatter light delivered by fiber optics to Avalanche Photo Diode detector arrays. Proprietary design ensures high performance, high efficiency, low-noise signal detection. Emission profiles are collected using reflective optics and single transmission band pass filters.

VIOLET SIDE SCATTER CONFIGURATION

Option to configure Avalanche Photo Diode detector array to collect side scatter signal from Violet (405 nm) laser. The configured channel (VSSC) can be used to better resolve nanoparticles.

QUALITY CONTROL

For detection channel off of the 405, 488, 561, and 638 nm laser, CytExpert QC automation pass/fail criteria is rCV ≤5.0%. For detection channels off of the 375 nm and 808 nm laser, the criteria is ≤ 7.0%.

PERFORMANCE

SCATTER RESOLUTION

Blue (488 nm) Side Scatter Resolution: <300 nm

Violet (405 nm) Side Scatter Resolution (VSSC):
80 nm polystyrene particles

Scatter performance is optimized for resolving human lymphocytes, monocytes, and granulocytes as well as nanoparticles.

CARRYOVER

Single Tube Format: ≤ 1.0%

Plate Loader Format: < 0.5%

SENSITIVITY

FITC: <30 molecules of equivalent soluble fluorochrome (MESF-FITC) from the 488 nm laser.

PE: <10 molecules of equivalent soluble fluorochrome (MESF-PE) from the 488 nm laser.

FLUORESCENCE RESOLUTION

The CytoFLEX Flow Cytometer is capable of achieving <3% rCV with alignment verification particles capable of rCVs <3%.

ELECTRONICS

NOMINAL ACQUISITION RATE

30,000 events per second with all configured parameters

Software capability to modify window extension parameter and to control abort rate during high event rate signal processing

SIGNAL PROCESSING

Fully digital system with 7 decade data display

SIGNAL

Pulse area, height for every channel, width for one selectable channel

FLUIDICS

Ultra-low pressure peristaltic sheath and sample delivery system

Sheath Fluid Filter and Sample Pump Tubing can be replaced by the user (no service visit required)

SAMPLE FLOW RATES

Fixed Flow Rates: 10, 30 and 60 µL/min

Custom Flow Rate Control mode from 10 to 240 µL/min in 1 µL increments.

FLUID CAPACITY

10L cubitainers

AUTOMATED MAINTENANCE FUNCTIONS

System Startup, Sample Mixing, Backflush, Prime, Shutdown, Deep Clean

SAMPLE INPUT FORMATS

5 mL (12 x 75 mm) polystyrene and polypropylene tubes

1.5 mL and 2 mL microcentrifuge tubes

PLATE LOADER FORMATS

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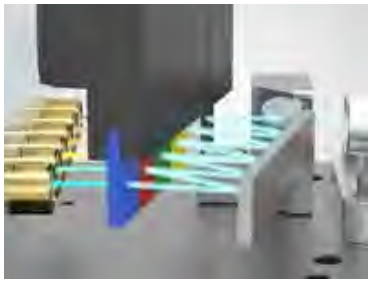
EXHIBIT 19

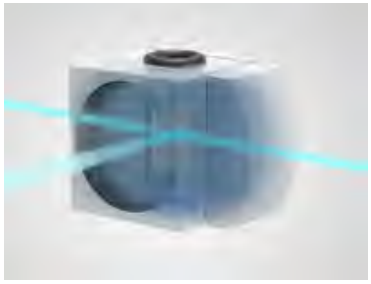
CytoFLEX Technology

A unique assembly of technologies contributes to the exquisite sensitivity of the platform



CytoFLEX Platform Wavelength Division Multiplexer WDM

A unique assembly of technologies contributes to the exquisite sensitivity of the platform. Borrowing technology from the telecommunications industry, the Wavelength Division Multiplexer (WDM) deconstructs and measures multiple wavelengths of light. The WDM relies on fiber optics and band pass filters to separate the light wavelengths. Unlike more traditional instruments, multiple dichroic filters to direct the light path are not required. This makes it much easier to configure the fluorescence channels, but also increases light efficiency as light loss due to refraction is minimized.

 *Avalanche Photodiode Detector from the CytoFLEX*	The WDM utilizes Avalanche Photodiode detectors (APD), versus Photomultiplier tubes (PMT). One hallmark of the photodiode is the high quantum efficiency in excess of 80%, especially for wavelengths greater than 800 nm.
 *Flow cell with integrated optics for flow cytometry*	With conventional analyzers, laser excitation sources are optimized by shaping and focusing light through a series of lenses and filters onto the flow cell. Each of these light interactions is an opportunity for light loss. Another component of the system which increases efficiency is the use of integrated optics to focus light onto the flow cell. All of these technologies work together in the CytoFLEX to ensure efficient light management for optimal excitation and emission of fluorochrome-tagged cells, which is critical to its performance.

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